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# FUNGAL COMMUNITY AND SOIL RESPONSES TO INVASIVE ALLIARIA PETIOLATA (GARLIC MUSTARD), SOIL WARMING, AND NITROGEN DEPOSITION

Mark Alan Anthony
*University of New Hampshire, Durham*

# FUNGAL COMMUNITY AND SOIL RESPONSES TO INVASIVE *ALLIARIA PETIOLATA* (GARLIC MUSTARD), SOIL WARMING, AND NITROGEN DEPOSITION

BY

MARK ALAN ANTHONY

BA, HAMPSHIRE COLLEGE, 2012

MS, UNIVERSITY OF NEW HAMPSHIRE, 2015

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Dissertation Director, Dr. Serita D. Frey,
Professor of Environmental Sciences

Dr. Kristina A. Stinson, Assistant Professor of Plant Ecology and Conservation, University of Massachusetts, Amherst

Dr. Richard G. Smith, Associate Professor of Cropping Systems Ecology and Management, UNH

Dr. Thomas D. Lee, Associate Professor of Natural Resources and the Environment, UNH

Dr. Thomas M. Davis, Professor of Plant Biology and Genetics, UNH

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**ABSTRACT**

FUNGAL COMMUNITY AND SOIL RESPONSES TO INVASIVE *ALLIARIA PETIOLATA* (GARLIC MUSTARD), SOIL WARMING, AND NITROGEN DEPOSITION

BY

Mark Alan Anthony

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Non-native, invasive plants can fundamentally alter soil fungal communities and edaphic properties. In this dissertation, I first measured fungal and edaphic responses and recovery to invasive, *Alliaria petiolata* (garlic mustard; Brassicaceae) eradication across Southern New England. I conducted an experimental eradication and tracked fungal communities and soil properties for three years in relation to reference uninvaded plots and actively invaded plots. Neither the fungal community nor soil properties recovered (*i.e.* became similar to reference uninvaded plots) following eradication, and I discuss how altered soil properties likely prevented fungal communities from recovering via environmental filtering. I also measured fungal and edaphic responses to the impacts of an experimental garlic mustard invasion in a long-term soil warming and simulated nitrogen deposition experiment. I found that soil warming had the strongest impact on fungal communities and warming interacted with invasion to amplify the impacts of invasion on fungal biomass and community composition. Garlic mustard, like all Brassicaceae, is non-mycorrhizal, and I characterized a mechanism that maintains the non-mycorrhizal status of these plants in a greenhouse study. The Brassicaceae produce secondary chemicals (glucosinolates) that deter herbivores and suppress plant pathogens, and I tested

whether these chemicals also prevent mycorrhizal fungi from colonizing Brassicaceae roots. I found that removing these chemicals from *Arabidopsis thaliana,* a related species to garlic mustard, increased mycorrhizal fungal colonization and suppressed plant performance in soils with mycorrhizal fungal inoculation, while mycorrhizal fungi did not affect plants that could produce these chemicals. Lastly, I re-analyzed publicly available fungal community data and mapped where across the globe fungi assemble in relation to different environmental conditions (*i.e.* floral, climatic, edaphic) versus dispersal and drift. Since extensive variation in fungal community composition cannot be explained by environmental variables in studies across the globe, I assessed whether certain regions of the world are more likely to structure fungal communities via niche or neutral processes. I found that in northern latitude forests and arctic tundra, fungal assembly was primarily due to niche processes. In most of the other plots across the globe fungal assembly was primarily stochastic and indicative of chronic dispersal and recruitment limitations. Together, the four studies in this dissertation offer new insight explaining how global change stressors and environmental filters affect fungal community composition.

## INTRODUCTION

There are circa 383,000 species of land plants (State of the World's Plants, 2017). While most of these inhabit a native range and provide fundamental natural resources, a small proportion (0.1%) get introduced into non-native ranges and become invasive (Williamson and Fitter, 1996). Invasive plants can imperil native plant diversity (Enloe *et al*. 2004, Stinson *et al*. 2006, Stromberg *et al*. 2009), soil microbial communities (Lankau, 2011, Lekberg *et al*. 2013, Anthony *et al*. 2017), and entire structures of food webs (David *et al*. 2017). Land owners and managers in the U.S. spend approximately $120 billion dollars each year managing and covering costs incurred from invasive species (Pimental *et al*. 2005). While invasive species are a current global change stressor, they may become more of an issue in the future since the number of plants introduced each year is steadily rising (Seebens *et al*. 2018). Climate change could also exacerbate plant invasions by expanding the geographic range of current invasive species (Bradley *et al*. 2010) and making ecosystems intrinsically more vulnerable to new invasions (Dukes and Mooney, 1999). Some of the most aggressive invasive species in the U.S. already occupy >1,000,000 ha **(**Blaustein, 2001, Rodgers *et al*. 2008), and they will likely never be eradicated completely (Rejmánek and Pitcarin 2002). Thus, it is important to understand the efficacy of regional management and to design adaptive management for future conditions.

Soil fungi are intimately involved in plant invasions, but they are generally not considered when managing invasive species. Soil fungi produce most of the enzymes that recycle plant materials and return organic nutrients back into the soil (Schneider *et al*. 2012). Invasive plants frequently increase soil nutrient cycling rates (Ehrenfeld, 2003) and the abundance and diversity of free-living saprotrophic fungi (Mincheva *et al*. 2014, Anthony *et al*. 2017). If saprotrophic fungi do not respond to invasive species removal then nutrient availability may

remain elevated, and this can maintain a higher likelihood for reinvasion (van Ruijven *et al*., 2003, Elgersma *et al*. 2012). Other types of fungi can also influence invasion. Many native plants, but not invasive plants, grow larger with mycorrhizal fungi (Klironomos, 2002), but the abundance and composition of mycorrhizal communities is sensitive to invasive plants (Barto *et al*. 2011, Lekberg *et al*. 2013). Different mycorrhizal communities can provide fewer benefits to native plants (Bever *et al*. 2009). Fungal pathogens are another guild that can accumulate in invaded soils (Eppinga *et al*. 2006, Anthony *et al*. 2017) and cause disease in native plants without harming invasive plants (Mangla *et al*. 2008). How fungi respond to invasive species eradication may therefore directly influence native plants recovery and the likelihood for reinvasion to occur.

At the moment, we are managing plant invasions without monitoring how soil fungal communities recover, but soil restoration is a holistic process that includes abiotic (e.g. pH, nutrient availability, soil carbon levels) and biotic (e.g. fungal biomass, diversity, composition) attributes, both of which may need to recover in order for an ecosystem to appear and function as it did prior to invasion (Bradshaw *et al*. 1996). A central goal of restoration ecology is to bring an ecosystem back to some desired state and frequently this is back to a familiar, uninvaded state (Stanturf *et al*. 2014, Török *et al*. 2018). This almost never happens since plant eradication efforts typically fail to completely remove an invasive species (Simberloff, 2009). A recent meta-analysis found that just 35% of invasive plant management initiatives produced a positive outcome for plant communities (Prior *et al*. 2018). Soil recovery (or lack thereof) to invasive plant management may help explain this poor success rate. There is evidence that invasive species eradication can restore certain fungal taxa in soils (Grove *et al*. 2012, Lankau *et al* 2014),

but to my knowledge no study has tracked how soil fungal and edaphic properties change together following invasive species management.

The experiments in this dissertation focus on *Alliaria petiolata* (Brassicaceae; garlic mustard), a non-native, invasive plant widely distributed across North America. Garlic mustard can invade temperate forest understories and is currently distributed throughout the entire temperate region of North America (USDA NRCS National Plant Data Team, 2018). The ecology of garlic mustard has been well described in two recent review articles (Rodgers *et al*. 2008, Cipollini & Cipollini, 2016), but the recovery of soils and their associated fungal communities in response to garlic mustard removal remains poorly understood. Fungal responses to management are especially important for garlic mustard invasions since garlic mustard suppresses mycorrhizal fungi and can transform the overall fungal community (Barto *et al*. 2011, Lankau, 2011, Lankau and Norduft, 2013, Anthony *et al*. 2017).

As a member of the Brassicaceae, garlic mustard is non-mycorrhizal and produces a suite of secondary chemicals that influence fungal communities (Roberts and Anderson, 2001, Callaway *et al*. 2008). These secondary chemicals are glucosinolates, which are produced by all Brassicaceae, and are well known for suppressing herbivores and fungi (Schreiner and Koide, 1993, Zukalová and Vašák, 2002). Garlic mustard extracts can also suppress arbuscular mycorrhizal fungal (AMF) and ectomycorrhizal fungal (EMF) germination and growth (Stinson *et al*. 2006, Wolfe *et al*. 2008, Cantor *et al*. 2011). Tree seedlings that have high mycorrhizal fungal dependency are less common in garlic mustard invaded areas because of loss and turnover in the mycorrhizal community (Stinson *et al*. 2007, Castellano and Gorchov, 2012). Across southern New England, the biomass of EMF is depleted in garlic mustard invaded forests and is replaced by free-living saprotrophic fungi and plant pathogens (Anthony *et al*. 2017). Soil

nutrient availability is twice as high in invaded compared to uninvaded areas (Rodgers *et al*. 2008, Anthony *et al*. 2017), soil pH becomes more basic (Anderson and Kelley, 1995), and soil C contents are reduced (Anthony *et al*. 2017), presumably because of accelerated decomposition in garlic mustard invaded soils (Rodgers *et al*. 2008). Abiotic and biotic soil properties are therefore highly responsive to garlic mustard invasion. The first chapter of this dissertation examines the response and recovery of soil fungi to multiple years of garlic mustard removal across Southern New England. The chapter builds on a baseline characterization of the impacts of garlic mustard invasion (Anthony *et al*. 2017) by evaluating the use of garlic mustard removal as a restoration tool.

The second chapter examines fungal responses to multiple global change stressors since forests in Southern New England are not only challenged by invasive species. Most notably, climate change has already caused the mean annual temperature of the Northeastern U.S. to warm by 0.75-1.5ºC compared to the preindustrial era (Halim *et al*. 2018). Climate change is also projected to increase the number and spread of non-native invasive species in the Northeast because invasive species in the south may expand their northern ranges (Bradley *et al*. 2010). At the same time, anthropogenic N deposition has been elevated in the Northeastern U.S. for decades (Li *et al*. 2016). The Clean Air Act (signed in 1963) has reduced the deposition of nitrate in the last two decades by 41%, but ammonium deposition, which is not regulated, has increased by 11% over the last 20 years (Li *et al*. 2016). The impacts of garlic mustard invasion could worsen with climate change and anthropogenic N deposition. The second chapter of my dissertation describes a study to evaluate the impacts of garlic mustard invasion in the context of long-term soil warming and simulated N deposition. Similar to Chapter one, I monitored the impacts of garlic mustard invasion on abiotic soil properties and soil fungal communities.

The third chapter tests a mechanism for the production of allelopathic chemicals produced by garlic mustard. Glucosinolates are produced by garlic mustard and can suppress mycorrhizal fungi on native plant roots (Roberts and Anderson, 2001, Stinson *et al*. 2006). While it is clear that glucosinolates are toxic to many types of fungi, saprotrophic and pathotrophic fungi do not seem to be as negatively impacted by garlic mustard invasion as compared to mycorrhizal fungi (Anthony *et al*. 2017). One simple explanation is that garlic mustard is non-mycorrhizal, so interference competition between garlic mustard roots and roots of native plants suppresses mycorrhizal fungi, but not free-living saprotrophic and pathotrophic fungi. However, glucosinolates are toxic to a broad class of fungi (including pathogens; Zhang *et al*. 2015) and even without garlic mustard present, extracts made from its biomass can suppress mycorrhizal fungal colonization (Stinson *et al*. 2006), though it is unclear how garlic mustard extracts affect other fungal guilds. In this chapter, I propose an alternative, novel explanation for the suppression of AMF.

Brassicaceae can be colonized by AMF even though they are non-mycorrhizal and generally lack genes that encode for mycorrhizal formation (Veiga *et al*. 2013, Delaux *et al*. 2013). AMF can also suppress Brassicaceae growth by >50% (Veiga *et al*. 2013, Fernandez *et al*. 2019). AMF and garlic mustard could mutually harm one another, though the potential negative impact of AMF on garlic mustard have not been addressed. In my third dissertation chapter, I examined whether glucosinolates suppress AMF colonization on non-host Brassicaceae roots. If garlic mustard actually produces glucosinolates to protect itself from AMF colonization, then the negative impacts it has on AMF could be related to defense rather than allelopathy. Since soil saprotrophic fungi and plant pathogens, which lack evolutionary history with garlic mustard, are presumably not trying to colonize garlic mustard roots, they may simply avoid physical contact

with most glucosinolates. This could help explain why these guilds are not negatively impacted by garlic mustard invasions (Anthony *et al*. 2017). While my third chapter is only loosely related to garlic mustard invasions, it provides a novel explanation for the ways Brassicacae maintain their non-mycorrhizal status. Other non-mycorrhizal plants have evolved to inhabit areas that generally lack AMF, but Brassicaceae share a similar niche to mycorrhizal vegetation and are chronically exposed to AMF (Lambers and Teste, 2013).

The fourth chapter in this dissertation addresses the broader processes that structure soil fungal communities and different soil fungal guilds. It is a re-analysis of multiple datasets on fungal communities spanning sites across the globe. In my earlier regional analyses of garlic mustard invasion, I found important roles for soil pH and C:N ratio to filter fungal communities independent of invasion (Anthony *et al*. 2017; Chapter 1 in this dissertation), and other studies report similarly important environmental filtering effects on fungal communities (Kivlin *et al*. 2014, Glassman *et al*. 2017), including at the global scale (Tedersoo *et al*. 2014, Davison *et al*. 2015). However there tends to be more residual variation in fungal community composition than variation explained by environmental parameters (Kivlin *et al*. 2014). This suggest that other processes unrelated to the environment also structure the fungal community. Neutral processes, such as random dispersal limitations and drift could be more important filters over fungal communities (Zhou and Ning, 2017), but both environmental filtering and neutral processes occur simultaneously (Hubbel, 2001, Nemergut *et al*. 2013), and we do not understand what influences the balance of these two processes. My fourth dissertation chapter therefore explored geographic, climatic, edaphic, and floral attributes associated with specific fungal assembly processes at a global scale.

# Chapter 1

## Fungal communities do not recover after removing invasive *Alliaria petiolata* (garlic mustard)

Anthony, M.A., Stinson, K.A., Trautwig, A.N., Coates-Connor, E., Frey, S.D.

(in review at *Biological Invasions*)

## 1. Abstract

The negative impacts of non-native invasive plants on native plants has prompted intensive eradication efforts, but whether eradication can restore soil microbial communities that are also sensitive to invasion is generally not considered. Some invasive plants, like *Alliaria petiolata* (garlic mustard), specifically alter soils in ways that promote the invasion process. Garlic mustard disrupts mycorrhizas, increases fungal pathogen loads, and elevates soil nutrient availability and soil pH; thus, the fungal community and soil property responses to garlic mustard eradication may be key to restoring ecosystem function in invaded forests. We conducted a garlic mustard eradication experiment at eight temperate, deciduous forests. One and three years after initiating annual garlic mustard removal (hand pulling), we collected soil samples and characterized fungal community structure using DNA metabarcoding alongside a suite of edaphic properties. We found that fungal richness, the number of shared fungal species, fungal biomass, and the relative abundance of fungal guilds became less similar to invaded plots by year three of eradication and more similar to uninvaded reference plots. However, fungal community composition did not resemble uninvaded communities by the third year of eradication and remained comparable to invaded communities. Soil chemical and physical properties also remained similar to invaded conditions. Overall soil abiotic-biotic restoration was not observed after three years of garlic mustard removal. Garlic mustard eradications may

therefore not achieve management goals until soil physical, chemical, and biological properties become more similar to uninvaded forested areas or at least more dissimilar to invaded conditions that can promote invasion.

## 1. Introduction

Non-native invasive species pose significant threats to biodiversity (Wilcove et al. 1998) and ecosystem function (Ehrenfeld, 2003) and are often intensively managed (Pimental et al. 2007, Corbin and D'Antonio, 2012). A central goal of managing biotic invasions is to bring community composition and function back to a set of desired reference conditions (Török et al. 2018). Most often this is back to an uninvaded state (Bradshaw, 1996, Stanturf et al. 2014). In practice, ecosystems may fully recover to an uninvaded state (restoration) or they may partially recover (rehabilitate), remain in an invaded state (no response), or turnover to form a third, novel state (replacement; Bradshaw, 1996). While there is evidence that management can rehabilitate or even restore native plant communities (Rejmánek and Pitcarin, 2002, Stinson et al. 2007, Simberloff, 2009, Pyšek and Richardson, 2010), few studies have addressed soil microbial responses to invasive plant management (Ried et al. 2009, Lankau et al. 2014). Microbes have been widely considered functionally redundant (Martiny et al. 2017, Louca et al. 2018) and so species membership has not been afforded the same attention as plants and animals. There are several benefits to considering microbial restoration in concert with plants and animals, especially fungal restoration (Heilmann-Clausen et al. 2015). Since fungi form mycorrhizas, cause disease as plant pathogens, and mediate nutrient cycling processes as decomposers, fungal restoration could have particular importance for native plant recovery (*e.g.* Stinson et al. 2006).

Invasive plants can strongly influence fungal communities. Some invasive plants produce allelochemicals that directly suppress fungi (Rodgers et al. 2008). Others are non-mycorrhizal

and can shift the dominant fungal trophic guild from mycorrhizal to saprotrophic (Pringle et al. 2009, Anthony et al. 2017). Some invasive plants also produce far more biomass with greater chemical complexity than native species litter, and this can reorganize saprotrophic communities (Mincheva et al. 2014) and alter soil carbon levels (Tamura and Tharyil, 2014). Despite well-documented changes in the fungal community in response to invasive species (Lankau, 2011, Lankau et al. 2014, Mincheva et al. 2014, Anthony et al. 2017), little work has addressed fungal recovery after invasive plant management. Those that have find only certain fungal species recover following eradication (Grove et al. 2012, Lankau et al. 2014). Plant species known to disperse efficiently can recover more quickly than dispersal limited species (Kiehl et al. 2010), but plants most likely to succeed in areas undergoing restoration are also those adjusted to the physical conditions of the eradicated area (Prach and Pyšek, 2001). Fungi have diverse dispersal capacities, and while many are efficient dispersers (Webster and Weber, 2007; Davison et al. 2015), others experience varying degrees of dispersal limitation (Peay et al. 2012; Glassman et al. 2017). Dispersal is an important aspect of community recovery, but fungal re-establishment may further vary depending on local edaphic conditions. The outcome of eradication may be especially variable across fungal guilds since saprotrophic, mycorrhizal, and pathotrophic fungi assemble differentially in relation to soil properties (Tedersoo et al. 2014). In this study, we tracked fungal communities and soil physical and chemical properties following *Alliaria petiolata* (garlic mustard) eradication and compared them to reference conditions in nearby uninvaded areas of the same forests. We worked at eight forests across southern New England where garlic mustard is well established in the forest understory (Rodgers et al. 2008), which allowed us to overcome the limitations of studying recovery at a single site and make broader

inferences about the recovery of soil fungi in response to eradication (*sensu* Stinson et al. 2007, Lankau et al. 2014).

Garlic mustard is a non-native, invasive forb in North America with well-known impacts on fungal communities and soil properties (Rodgers et al. 2008). We have previously found that garlic mustard invasion is associated with depleted mycorrhizal fungal biomass, and a shift towards increased dominance by saprotrophic and pathogenic fungi known to influence biogeochemical cycles and native plant health (see Anthony et al. 2017). Garlic mustard invasion can also accelerate decomposition and nutrient cycling (Rodgers et al. 2008), decrease soil carbon concentrations (Anthony et al. 2017), and increase soil pH (Anderson and Kelley, 1995, Rodgers et al. 2008, Anthony et al. 2017). Garlic mustard has invaded 37 states in the U.S.A. and is found throughout southern Canada (USDA NRCS National Plant Data Team, 2018). Given the extensive introduced range of garlic mustard, complete eradication is not realistic, but garlic mustard removal initiatives are common (*e.g.* The Stewardship Network Garlic Mustard Challenge). At the moment, land managers are removing garlic mustard from the landscape without knowing if this promotes soil restoration, or whether restoration is consistent across geographic scales. We have already found that eradicating *A. petiolata* can increase native plant diversity and tree seedling abundances (Stinson et al. 2007) and reduce non-native earthworm abundances (Stinson et al. 2018), forming communities that mirror those without a history of invasion. The objectives of this study were therefore to characterize fungal communities and soil properties from invaded and post one- and three-year eradication areas in comparison to adjacent uninvaded reference areas in multiple forested locations.

## 1. Methods

### *1. Sites and study design*

This work was conducted at eight temperate, deciduous forests in southern New England, U.S.A., a detailed description of which can be found in Anthony et al. (2017). Briefly, the overstory at all sites is of mixed composition with maple (*Acer saccharum, A. rubrum*), oak (*Quercus rubra*), ash (*Fraxinus americana*), and white pine (*Pinus strobus*) canopy trees. Tree seedlings, Canada mayflower (*Mianthemum candense*), trout lily (*Erythronium americanum*), and jack-in-the-pulpit (*Arisaema triphyllum*) are the most abundant understory vegetation. Within each site, we established replicate 3 × 3 m plots in June 2013 to be able to compare uninvaded, invaded, and 'to-be' eradicated plots. All invaded plots had garlic mustard at densities >20 plants $m^2$ at the time of establishment. Each plot was separated by at least 10 m, with replicate uninvaded, invaded, and eradicated plots paired based on similar native understory vegetation, slope, aspect, and relief. Despite pairing plots across invasion statuses in order to account for site level heterogeneity, we cannot discern whether invaded and 'to-be' eradicated plots were similar to reference uninvaded plots prior to being invaded. We began removing all garlic mustard by hand pulling from the eradicated plots in May, 2014. We maintained eradication thereafter on an annual basis. In total, there were three replicate uninvaded, invaded, and eradicated plots at each forest (8 sites × 3 invasion statuses × 3 replicates = 72 plots).

*1. Soil sampling, processing, and analyses*

We collected soil samples from each plot one (June 2015) and three (June 2017) years after starting the eradications. The organic horizon was sampled by removing three intact 10 × 10 cm of the forest floor to the depth of the mineral soil surface (~3-5 cm). Mineral soil was collected to a depth of 5 cm beneath each organic horizon sample using a 5 cm diameter sledge-hammer soil corer. Samples from each plot were homogenized by depth for a total of 144 samples and stored in a 4°C cooler in the field until being processed within 24 hours.

All soil was passed through a 2 mm sieve to remove rocks, roots, and coarse organic debris >2 mm. A soil subsample (~5 g) was immediately frozen at -80°C for molecular analysis and another (~10 g) at -20°C for microbial biomass assessment via phospholipid fatty acid (PLFA) analysis. Within 48 hours of sampling, we measured gravimetric soil moisture after drying at 60°C for 48 hours. Bulk density was estimated as the mass of dry soil after correcting for the mass of rocks divided by the volume of collected soil. Soil pH was determined using air-dried soil and distilled water (1 g : 10 mL). Total organic C and N was measured on air-dried, finely ground soil using dry combustion on a Perkin Elmer 2400 Series II CHN elemental analyzer (Waltham, MA). The remaining soil (10 g) was extracted for inorganic N using 2M KCl (40 mL) and analyzed for ammonium and nitrate using a colorimetric microplate assay (Braman and Hendrix, 1989).

Microbial biomass was estimated on freeze-dried soil (Freezone 6, Labconco, Kansas City, MO) using PLFA analysis. Briefly, lipids were extracted from soil (1 g) using phosphate buffer, chloroform, and methanol (0.8:1:2; v:v:v). The polar lipids were isolated using silicic acid chromatography and then methylated using 0.2M methanolic potassium hydroxide (1 mL) and incubating at 60°C for 30 minutes to form fatty acid methyl esters (FAMES). The FAMES were dried and reconstituted in hexane for quantification on a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (Palo Alto, CA). We compared FAME peaks against a standard library of FAMES specific to fungi (18:2ω6, 9c, 18:1ω9c) (Matreya, LLC, Pleasant Gap, PA). A standard control biomarker (c19:0) was used to convert peak area concentrations into nmol PLFA $g^{-1}$ dry soil.

Fungal community structure (richness and community composition) was characterized using ITS2 metabarcoding on the Illumina MiSeq platform. DNA was extracted from soil (0.25

g) using the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). The ITS2 region was amplified using the fungal specific primer pair fITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1990). PCR primers contained the Illumina adaptor sequence, an 8 bp pad sequence, a 2 bp linker sequence, and were dual indexed to include two unique 8 bp sequences (see custom PCR primer constructs, Supplementary Table 1.1). PCR reactions were performed in triplicate for each sample in 25 µL reactions with the following reagents: PCR Grade $H_2O$ (13 µL), PCR master mix (10 µL; Phusion® High Fidelity Master Mix, New England Biolabs, Ipswitch, MA), 10 µM fITS7 (0.5 µL), 10 µM ITS4 (0.5 µL), and template DNA (1 µL). Thermocycler conditions followed that of Anthony et al. (2017). PCR products were cleaned using the AxyPrep MAG PCR Clean-up kit (Corning, Tewksbury, MA). Final PCR products were inspected on an agarose gel and DNA concentration was measured by fluorometry on a Qubit® 3.0 Fluorometer (Life Technologies, Grand Island, NY). Equimolar libraries of the samples were split on two separate Illumina MiSeq runs (2 x 250 bp chemistry) for each sampling year for a total of four MiSeq runs at the Center for Genomics and Bioinformatics at Indiana State University (Bloomington, IN). Raw sequences were deposited in the NCBI Sequence Read Archive under bioproject number PRJNA504442.

Sequences were passed through a series of quality control measures. First, we removed low quality bases and reads (Phred score < 2), short reads (< 100 bp), and Illumina adapter and PCR primer sequences using Trimmomatic (v0.26-0.32; Bolger et al. 2014). We then merged forward and reverse reads at a 20-50 bp overlap allowing 5% mismatch using the join_paired_ends.py function in QIIME and the fastq_mergepairs function in USEARCH (v11; Edgar, 2010) for the one and three-year samples, respectively. Next, we isolated the ITS2 fragment from each sequence using the ITSx software (v1.011; Bengtsson-Palme et al. 2013).

We used the USEARCH (v8 and v11; Edgar, 2010) pipeline to create OTU tables. We dereplicated the sequences and excluded sequences <150 bp (derep_fullllength). Then we sorted the sequences and removed singletons (sortbysize), and calculated sequence similarity at a 97% cutoff while removing chimeras (cluster_otus). We assigned taxonomy to OTUs using the UNITE reference database (version 7; January 2016 release) and the utax reference database (January 2017 release) using the assign_taxonomy.py function in QIIME and the Sintax approach in USEARCH for the one- and three-year analyses, respectively. OTUs without a match to fungi were blasted against the entire NCBI *nr* database, and OTUs assigned to non-fungal organisms were removed from subsequent analyses. Genus-level taxonomic assignments were compared to the FUNGuild database to make functional guild annotations (Nguygen et al. 2016) and all 'probable' or above probable matches were included for subsequent analyses. We grouped fungi by trophic mode (saprotrophic, pathotrophic) and whether they were ectomycorrhizal fungi (EMF). Arbuscular mycorrhizal fungi were not included in functional guild analyses because the primers we used poorly target the Glomeromycotina. A table of sequence retention after quality control steps, taxonomic annotation, and guild annotations is found in the supplementary materials (Supplementary Table 1.2).

*1. Statistical Analyses*

All statistical analyses were conducted in R 3.0.2 (R Development Core Team, 2008), with significance across all tests set at $P \leq 0.05$. Linear mixed effects models were used to look for significance of univariate response variables (*i.e.,* fungal richness, biomass, soil properties) across sites, invasion statuses, and site × invasion statuses using the lme function within the nlme package (Pinheiro et al. 2007). We created beyond optimal models that parameterized for autocorrelation and unequal variance across predictor variables. We used *t*-tests with

heteroscedastic variance to make multiple comparisons. All of the edaphic properties were also analyzed together using Euclidean distance and principle correspondence analysis (PCoA) with the pcoa function in the ape package (Paradis et al. 2004). Fungal community analyses were run after randomly rarifying the OTU table 1,000 times at the lowest sequencing depth of 4,000 and 1,025 sequences per sampling unit using the rrarefy function for the one- and three-year samples, respectively. Species (OTU) richness was calculated using the specnumber function within the vegan package (Oksanen et al. 2013). The number of shared OTUs (species overlap) among invasion statuses was calculated using the ChaoShared function within the SpadeR package (Chao et al. 2016). Multivariate analyses of fungal community composition were run using resemblance-based permutation methods. Permutation ANOVA (PERMANOVA; Anderson, 2001) and heterogeneity of multivariate dispersion (PERMDISP; Anderson et al. 2006) were run using the functions adonis and betadisper in the vegan package. Heterogeneity of multivariate dispersion is a measure of beta diversity. Communities are divergent if community composition is variable across space (high beta diversity) and convergent if composition is similar across space (low beta diversity; Anderson et al. 2006). Distance-based analyses for fungi were performed on Bray-Curtis dissimilarity matrices calculated from OTU relative abundances. Significance of permutation methods was determined after 1,000 permutations. Different visualization approaches were used depending on whether one versus two dimensions we were being analyzed. Non-metric multidimensional scaling (NMDS) was used to visually display fungal community composition using the metaMDS function (vegan) since NMDS does not use eigenvalues (*sensu* PCoA) and is most appropriate for visualizing the highest amount of variation in communities using two dimensions. We used PCoA axes to account for variation in fungal community composition based on a single axis since PCoA decomposes variation to many

individual axes (unlike NMDS). To compare abiotic-biotic soil recovery pathways, we analyzed co-variation between the first PCoA axis (most explanatory) of all soil properties (Euclidean distance) and the first PCoA axis of fungal community composition (Bray-Curtis).

## 1. Results

### *1. Soil physical and chemical properties and fungal biomass*

Soil pH and inorganic N contents were elevated in association with invasion and remained higher in the eradicated compared to uninvaded reference plots (Table 1.1). By year three, soil pH in eradicated plots was in between that of the uninvaded and invaded plots. Soil C-stocks and soil C:N ratio tended to be lower in invaded and eradicated plots compared to uninvaded reference plots, but this was only significant in year three soils in the organic and mineral soils, respectively. Overall, fungal biomass varied across invasion statuses in year one ($F_{(2,94)}$; $P$ = 0.01) but not in year three ($F_{(2,91)}$, $P$ = 0.61; Supplementary Table 1.3). Specifically, the eradicated plots had higher fungal biomass than the invaded plots in year one (*t*-test; $P$ = 0.005), but this disappeared by year three. There were otherwise no differences in fungal biomass.

### *1. Fungal richness, evenness, species overlap, and community composition*

Fungal richness was elevated in association with invasion ($F_{(2,90)}$ = 8.5, $P$ = 0.004) and remained elevated in the eradicated plots one year after eradication (Supplementary Table 1.4). The eradicated and invaded plots also shared 484 OTUs while eradicated and uninvaded reference plots only shared 246 OTUs (Supplementary Fig. 1.1). By the third year of eradication, species membership became less similar to invaded plots. Fungal richness was no longer elevated in the eradicated plots compared to uninvaded plots (*t*-test; $P$ = 0.32; Supplementary Table 1.4), and eradicated and invaded communities only shared 217 OTUs while approximately the same number of OTUs (240) were shared with uninvaded plots as in year one. In contrast to

the number of shared OTUs, community composition differed between uninvaded reference plots and the invaded and eradicated plots in both years (PERMANOVA: Table 1.2; Fig. 1.1), while eradicated and invaded communities were not different from each other in either year. Specifically, variation in community composition was lower in both the invaded and eradicated plots compared to uninvaded reference plots (PERMDISP: Table 1.2).

*1. Guild relative abundances*

EMF relative abundance was lower while saprotrophic and pathotrophic fungal relative abundances were higher in the invaded compared to uninvaded plots (Fig. 1.2). After one year of eradication, the relative abundances of EMF, saprotrophic, and pathotrophic groups were similar between eradicated and uninvaded reference plots. After three years of eradication, there were no differences between uninvaded, invaded, and eradicated plots in terms of guild relative abundances. In contrast, the taxonomic composition of functional guilds varied across invasion statuses in year one and in year three. In year one, there were reduced relative abundances of some EMF taxa (Russulales, Sebacinales, Cantharellales) and increased relative abundances of some saprotrophs (Mortierellomycetes, Sordariomycetes, Wallemiomycetes) and pathotrophs (Polyporales, Urocystidales, Olpidiales) in the invaded plots compared to uninvaded plots (Table 1.3). The relative abundance of certain EMF (Russulales and Sebacinales) increased to levels between uninvaded and invaded plots in the eradicated plots while other EMF had higher relative abundances in the eradicated plots compared to reference uninvaded plots (Agaricales, Pezizales). Some of the same saprotrophs (Wallemiomycetes, Basidiobolales) and pathotrophs (Polyporales, Urocystidales, Olpidiales) had similarly high relative abundances in eradicated plots as in invaded ones, in addition to different saprotrophs (Basidiobolales) and pathotrophs (Spizellomycetales). In the year three sampling, taxonomic composition still varied among all

three invasion statuses (Table 1.3). Notably, there was still higher relative abundances of pathotrophic Urocystidales and Olpidiales in the invaded and eradicated plots compared to uninvaded plots.

*1. Relationships between environmental variables and the fungal community*

Shifts in the fungal community were correlated to soil properties and may have prevented overall abiotic-biotic soil system recovery. First, variation in fungal community composition was restricted (*a.k.a.* converged) in association with invasion and eradication (Fig. 1.1), and the degree of community convergence (i.e. decrease in fungal beta diversity) was positively correlated to soil pH (Fig. 1.3; $R^2 = 0.48$, $P < 0.0001$). Overall, when we examined variation in fungal community composition (biotic) relative to all abiotic soil variables, soil systems in the eradicated treatments were not different from invaded soil systems (Fig. 1.4). This indicates that eradication did not restore soil systems back to an uninvaded state, despite partial rehabilitation in fungal richness, community evenness, species-overlap, and total fungal guild relative abundances.

## 1. Discussion

Extensive research and conservation initiatives have sought to understand and control non-native invasive species (Simberloff, 2009). Despite this attention, invasion ecology has largely overlooked what happens in soils after an invasive plant has been removed (Lankau et al. 2014), even though mycorrhizas and pathogens (Bennet et al. 2017) and soil fertility (Meekins and McCarthty, 2000) all effect plant regeneration and growth. If soil physical, chemical, and biological conditions remain similar in eradicated compared to invaded areas, then sites undergoing restoration may be prone to re-invasion and require additional management in the future (Ries et al. 2004). To that end, our most important findings were that fungal richness,

species overlap, and functional guild relative abundances partially recovered to reference conditions after three years of garlic mustard removal, while fungal community composition and soil chemical and physical attributes remained fundamentally different in eradicated relative to uninvaded reference plots. Since fungi differentiating uninvaded and eradicated plots have evolved different ecological strategies, the functioning of areas undergoing eradication has likely not recovered to an uninvaded state. Further, soil pH, inorganic N concentrations, and soil C stocks remained altered in the eradicated plots, likely prohibiting fungal community restoration via environmental filtering (Kivlin et al. 2014). Although we hypothesize that some ecosystem functions in the eradicated plots have been restored due to increased total ectomycorrhizal and reduced total pathotrophic fungal abundances, overall soil abiotic-biotic recovery to uninvaded reference conditions was not responsive to three years of annual garlic mustard removal.

1. *Soil properties did not recover*

Garlic mustard invasion has well-known impacts on soil properties (Rodgers et al. 2008, Anthony et al. 2017), but whether soil properties respond to garlic mustard eradication is unclear. By the third year of eradication in our study, eradicated plots had higher ammonium and nitrate contents and reduced organic soil C-stocks and mineral soil C:N ratios compared to uninvaded plots. Soil pH decreased to be in between uninvaded and invaded levels, but it was still elevated following eradication. It is worth noting that differences in soil properties across invasion statuses were similar across years despite some fluctuations in actual soil property values. In particular, first year samples had higher soil pH and nitrate contents than we observed in our base-line analysis (Anthony et al. 2017) and compared to year three (Table 1.1). We think this may have to do with a drought in 2015 (mean annual precipitation: 102 cm) compared to our baseline data collected in 2013 (116 cm) and data collected in 2017 (115 cm) (Northeast RCC,

2018). Despite fluctuations in actual edaphic values, soil pH and nitrate contents were still elevated in the invaded and eradicated plots in both years. Three years of garlic mustard removal therefore did not restore soil chemical and physical properties back to reference uninvaded levels.

1. *Restoration of fungal richness but not community composition*

Fungal richness was elevated in the invaded plots prior to eradication (Anthony et al. 2017) but decreased to uninvaded levels after three years of eradication. Our results are consistent with another study showing that arbuscular mycorrhizal fungal richness was restored to uninvaded levels after six years of garlic mustard eradication (Lankau et al. 2014). This provides strong evidence that garlic mustard invasions are responsible for changes in fungal richness, either directly, as has been suggested before (Lankau, 2011, Anthony et al. 2017) or indirectly through changes in plant communities (Stinson et al. 2007) and earthworm densities (Stinson et al. 2018), but we cannot discern what the composition of fungal communities was like in the invaded and eradicated plots prior to invasion since this is a correlative study. Restoration of fungal richness is only indicative of potential fungal establishment, however, since it does not consider abundance (*i.e.* community composition). If fungi are like plants, then fungi must colonize and increase abundances dramatically to become viable community members (Williamson and Fitter, 1996, Tilman, 2004). Community composition, measured as Bray-Curtis dissimilarity, measures species membership alongside relative abundances. It remained altered in the eradicated plots, indicating that soil fungal communities have not been fully restored to uninvaded reference plots. There are therefore other elements of garlic mustard invasion beside the presence of garlic mustard that influence fungal establishment and persistence (*i.e.* legacy effects, soil properties).

We previously found that garlic mustard invasion was associated with lower regional beta diversity since fungal communities in invaded plots converged towards a common composition (Anthony et al. 2017), and we found in our current study that the fungal beta diversity remained lower in the eradication treatments. Communities can converge towards a common community composition when environmental variation is low (Caruso et al. 2012). Since soil properties in garlic mustard invaded soils are more homogenous than uninvaded soils (Anthony et al. 2017), and there was little restoration of soil properties in the eradicated plots, we suggest that community homogenization is related to low environmental variation. In particular, soil pH remained elevated across the eradicated plots compared to the uninvaded plots. We also found that the degree of convergence in community composition was positively correlated to soil pH (Fig. 1.3). Elevated soil pH in the eradicated and invaded plots likely contributed to homogeneity of fungal communities. Since soil properties have well known environmental filtering effects on fungi (Kivlin et al. 2014, Glassman et al. 2017), there will likely be legacy effects of invasion on fungal community composition as long as soil properties remain altered. However, it is important to acknowledge the correlative nature of our study since we do not know what the soil conditions in invaded and eradicated plots were prior to invasion.

1. *Fungal guild relative abundance but not taxonomic membership and relative abundance were restored*

Invasive plants leave a signature in the microbial community (legacy effect) even after their removal (Elgersma et al. 2011, Grove et al. 2012), but relatively little is known about which fungal groups or individual taxa are responsible for these legacy effects (Lankau et al. 2014). We found that garlic mustard invasion decreased EMF relative abundance but increased that of saprotrophs and pathotrophs, mirroring our earlier results comparing uninvaded and invaded

communities (Anthony et al. 2017). Eradication appears to return total guild relative abundances back to uninvaded reference levels within the first year of eradication, with an increase in EMF and decreases in saprotrophic and pathotrophic relative abundances. There was generally no impact of invasion or eradication on fungal biomass, with one exception. There was higher fungal biomass in the eradicated plots after the first year compared to invaded plots. Since EMF increased in relative abundance in the eradicated compared to invaded plots, we hypothesize that the temporal increase in fungal biomass was due to initial recolonization by EMF. There were EMF taxa that increased in relative abundance in the eradicated compared to invaded plots in the first year that were not significantly more abundant by the third year (Agaricales and Cantharellales). Of highest relative abundance were EMF Agaricales, including *Amanita, Gliophorus, Hebeloma, Hymenogaster, and Inocybe* (Supplementary Table 1.5). Differences in fungal traits, including growth rates and dispersal mode, may make certain taxa especially effective at initial recolonization (Twieg et al. 2007, Moeller et al. 2014). For example, *Amanita* produces larger mushrooms than most EMF and has high spore production (Bässler et al. 2015), while members of the *Hebeloma*, *Hymenogaster*, and *Inocybe* genera have ornamented spores that may promote dispersal by animals (Güler and Türkoglu, 2015, Halbachs et al. 2015).

At the third year of eradication, fungal biomass returned to uninvaded reference levels and was also not different from invaded plots. The relative abundance of EMF Agaricales was still higher in the eradicated plots, but this was no longer significant. Rather, the EMF community was depleted in Mytilinidiales (*Cenococcum*) and Gomphales (*Ramaria*). The loss of *Cenococcum* is especially concerning because this taxon was previously identified as an indicator taxon of uninvaded soils and sensitive to garlic mustard invasion (Anthony et al. 2017). *Cenococcum* is also resistant to abiotic stressors and may be especially important during times of

drought (Fernandez and Koide, 2013). Ectomycorrhizas without *Cenococcum* may be particularly vulnerable to climatic stressors, especially in garlic mustard invaded forests. In addition to the EMF that were not present in the eradicated plots, there were also those that recovered by year three. The most abundant EMF lineage, the Russulales, comprised of *Russula* and *Lactarius,* were one such group. *Russula* and *Lactarius* are known to have ligninolytic decomposing abilities (Looney et al. 2018), and they are often found between decomposing leaves and root tips, so they are especially beneficial symbionts for nutrient acquisition (Agerer, 2001). Since EMF Russulales are also the dominant EMF across the study sites, recovery of these fungi should benefit ectomycorrhizal tree seedlings that are vulnerable to garlic mustard invasions (Stinson et al. 2007, Castellano and Gorchov, 2012). Overall, we found that EMF have been partially rehabilitated since some taxa have recovered while others remain in an altered state.

Saprotrophic fungi also exhibited partial recovery to garlic mustard eradication with sustained alteration to some taxa. We have previously found that garlic mustard invasion associated with increased relative abundance of Mortierellomycetes (Anthony et al. 2017) and they were still at higher relative abundances in the eradicated plots in our current study. At year three, there were reduced relative abundances of Geoglossomycetes (*Geoglossum*, *Glutinoglossum*, *Trichoglossum*) but increased relative abundance of Umbelopsidomycetes (*Umbelopsis*) in the eradicated plots. It is notable that none of the saprotrophic fungi that had different relative abundances in the eradicated plots were Basidiomycota. Saprotrophic Agaricomycetes (Basidiomycota), which include the strongest lignocellulose decomposers (Floudas et al. 2012), were comparable between uninvaded and eradicated plots. We therefore suspect the same potential for leaf litter decomposition between uninvaded and eradicated plots

but differing organic matter decomposition potential due to sustained alteration to the Mortierellomycetes, Geoglossomycetes, and Umbelopsidomycetes.

We also found that pathotrophic fungi partially recovered following garlic mustard eradication. There were two classes of pathogens that were only found in the invaded and eradicated plots (pathogenic Eurotiales and Olpidiales). The Eurotiales included 11 *Penicillium* species, *Aspergillus citriporus, Sagenomella diversispora*, and three *Talaromyces* species with varying animal and plant virulence. Of particular interest, we found that the sole member of the Olpidiales, *Olpidium brassicae* was never found in the uninvaded plots, but it was present across the invaded and eradicated plots. We previously reported that *Olpidium brassicae* was exclusively found in garlic mustard invaded plots (Anthony et al. 2017), and so this taxon is a stable member of the invaded landscape and does not go locally extinct with garlic mustard eradication. *O. brassicae* can transmit plant viruses that spill over into neighboring plants, but this fungus is not known to cause disease in the host plant (Hartwright et al. 2010. *O. brassicae* also produces zoospores that can remain dormant for up to 20 years (Campbell, 1985). It is also important to acknowledge that despite these fungi being annotated as pathotrophs, it is possible that they are actually living as saprotrophic fungi. We cannot discern the ecology of pathotrophic fungi with flexible trophic modes based on DNA metabarcoding. Nonetheless, the prevalence of novel pathotrophic fungi is consistent with earlier work (Anthony et al. 2017) and could have negative impacts on plant and soil animal recovery.

We previously found a shift in the dominant trophic guild from EMF in reference uninvaded forested areas to saprotrophic and pathotrophic fungi with garlic mustard invasion (Anthony et al. 2017). We found the same pattern in the first-year samples of this current study, but this was gone by year three. In the first year, the average cover of adult garlic mustard was 14 plants $m^2$

(5% relative abundance), while in the third year it was 6 plants $m^2$ (3% relative abundance). Since adult plants are larger and produce more of the secondary chemicals than first year plants (unpublished data; Supplementary Fig. 1.2), decreased adults in the third year could allow the fungal community 'to recover' in the invaded plots. Other work has also shown that the density of garlic mustard scales with the impacts of invasion on AMF richness (Lankau et al. 2011) and AMF community composition (Burke et al. 2011). Since garlic mustard invasions tend to be patchy across an invaded forest, it is likely that times of low garlic mustard cover result in rapid recovery of fungal guild relative abundances. This could be a potentially good time to introduce native species, especially ectomycorrhizal tree seedlings that rely more heavily on mycorrhizas (Bennet et al. 2017).

**1. Conclusion: abiotic-biotic soil recovery**

Remediation should consider how both abiotic (*e.g.* edaphic properties) and biotic (*e.g.* community composition) attributes change (or not) in concert during restoration. Soil abiotic and biotic elements shape each other through environmental filtering and microbial metabolism, and this system informs overall response to management (Bradshaw, 1996). We applied this framework to our study and found that eradicated sites did not recover or even rehabilitate after three years of garlic mustard removal (Fig. 1.4). Rather, eradicated sites remained indistinguishable from invaded ones. Of course, there are many abiotic and biotic elements that we did not measure, but basic soil properties and fungal communities are good indicators of soil function in temperate forests. We therefore conclude that garlic mustard management in southern New England should anticipate eradication programs requiring longer than three years to restore soils. Work on arbuscular mycorrhizal fungi has shown that even after six years, AMF communities do not fully recover (Lankau et al. 2014). Environmental stressors can permanently

alter soil systems (Bradshaw and Chadwick, 1980), but we do not know what the long-term impacts of invasion are on soils. Future work will need to track the long-term (>10 years) outcomes of eradication in order to determine if restoration is possible. We also think future work should address whether eradicated areas are equally prone to invasion as uninvaded or currently invaded areas, whether tree seedlings survive as well in the eradicated landscape despite different fungal taxonomic makeup (including AMF species that have not yet been studied in detail or across multiple forested areas), and whether eradicated soils have the potential to start storing soil C again. These important questions can help land managers determine whether the eradicated landscape, despite not being restored, adequately supports native biodiversity and ecosystem function.

**Table 1.1.** Table of soil variables. Values represent the mean, with standard errors shown in parentheses. Values with different lowercase letters are significantly different across invasions statuses in the organic horizon and mineral soil, respectively.

| | | Soil pH | Ammonium ($\mu g\ g^{-1}$ dry soil) | Nitrate ($\mu g\ g^{-1}$ dry soil) | Soil C ($g\ C\ m^{-2}$) | Soil C:N ratio | Fungal biomass (nmol PLFA $g^{-1}$ dry soil) |
|---|---|---|---|---|---|---|---|
| **One year** | | | | | | | |
| *Organic* | Uninvaded | 4.9 (0.17)a | 21.6 (3.5)a | 66.0 (18.1)a | 18.08 (1.78)a | 13.1 (0.85)a | 66 (7)ab |
| | Invaded | 5.5 (0.13)b | 17.3 (2.4)a | 143.5 (40.3)b | 17.55 (1.83)a | 13.57 (0.83)a | 56 (5)b |
| | Eradicated | 5.3 (0.14)b | 22.5 (5.2)a | 185.9 (35.7)b | 17.21 (1.45)a | 13.97 (0.27)a | 79 (7)a |
| *Mineral* | Uninvaded | 5.0 (0.13)a | 21.4 (3.5)a | 56.7 (10.5)a | 18.7 (1.21)a | 11.66 (1.14)a | 42 (6)a |
| | Invaded | 5.2 (0.11)b | 13.2 (1.9)b | 58.7 (15.8)a | 16.85 (1.81)a | 12.11 (0.77)a | 35 (4)a |
| | Eradicated | 5.1 (0.1)ab | 19.1 (3.1)ab | 42.7 (12.8)a | 18.84 (2.39)a | 12.48 (0.8)a | 41 (4)a |
| **Three** | | | | | | | |
| *Organic* | Uninvaded | 4.3 (0.17)a | 8.3 (1)a | 0.3 (0.1)a | 20.94 (2.33)a | 15.09 (0.51)a | 68 (6)a |
| | Invaded | 4.7 (0.16)b | 13.0 (3)ab | 0.6 (0.1)b | 17.37 (2.02)b | 14.18 (0.27)a | 68 (7)a |
| | Eradicated | 4.4 (0.17)ab | 10.9 (1)b | 0.7 (0.1)b | 18.98 (2.82)b | 14.17 (0.38)a | 73 (6)a |
| *Mineral* | Uninvaded | 4.2 (0.16)a | 5.9 (0.5)a | 0.3 (0.1)a | 21.87 (2.45)a | 14.66 (0.51)a | 33 (2)a |
| | Invaded | 4.6 (0.12)b | 8.5 (1.07)b | 0.3 (0.1)a | 16.48 (2.06)a | 12.85 (0.42)b | 33 (3)a |
| | Eradicated | 4.4 (0.11)ab | 7.5 (1.07)ab | 0.3 (0.1)a | 17.94 (1.37)a | 13.32 (0.41)b | 36 (4)a |

**Table 1.2.** Table of statistical values showing the influence of site, invasion, soil horizon, and site × invasion on fungal community composition (PERMANOVA) and variation in heterogeneity of community composition (PERMDISP). Analyses were performed on Bray-Curtis dissimilarities, and significance was determined after 1,000 permutations. Dashes indicate where a statistical output was not generated. Unv., Erad., and Inv. is short for uninvaded, eradicated, and invaded, respectively.

| | One year | | | Three years | | |
|---|---|---|---|---|---|---|
| | *F*- | $R^2$ | *P*-value | *F*-value | $R^2$ | *P*-value |
| PERMANOVA | | | | | | |
| Site | 4.95 | 0.19 | **0.001** | 2.21 | 0.14 | **0.001** |
| Invasion | 2.39 | 0.03 | **0.001** | 1.**52** | 0.02 | **0.001** |
| Unv. × Erad. | 1.92 | 0.02 | **0.001** | 1.55 | 0.02 | **0.002** |
| Unv. × Inv. | 1.94 | 0.02 | **0.001** | 1.63 | 0.02 | **0.001** |
| Erad. × Inv. | 1.07 | 0.01 | 0.27 | 1.05 | 0.01 | 0.33 |
| Horizon | 2.11 | 0.01 | **0.001** | 1.78 | 0.01 | **0.001** |
| Site × Invasion | 1.62 | 0.03 | **0.03** | 0.01 | 0.12 | **0.001** |
| PERMDISP | | | | | | |
| Site | 2.94 | - | **0.007** | 31.3 | - | **< 0.0001** |
| Invasion | 9.61 | - | **0.0001** | 6.65 | - | **0.002** |
| Unv. × Erad. | 7.27 | - | **0.008** | 12.76 | - | **0.001** |
| Unv. × Inv. | 17.5 | - | **<0.0001** | 9.69 | - | **0.004** |
| Erad. × Inv. | 3.49 | - | 0.07 | 0.11 | - | 0.74 |
| Horizon | 0.26 | - | 0.61 | 8.29 | - | **0.005** |
| Site × Invasion | 1.37 | - | 0.14 | 12.5 | - | **<0.0001** |

**Table 1.3.** The relative abundance of ectomycorrhizal (EMF; orders), saprotrophic (classes), and pathotrophic (orders) taxonomic relative abundances that were significantly altered by invasion status one and three year after eradication. Values are the mean and error bars are the standard error. Values with different lowercase letters within a lineage are significantly different.

| **Year one** | | **Uninvaded** | **Invaded** | **Eradicated** |
|---|---|---|---|---|
| **EMF** | Russulales | 0.147 (0.027)a | 0.064 (0.016)b | 0.105 (0.024)ab |
| | Agaricales | 0.036 (0.01)ab | 0.02 (0.008)a | 0.055 (0.015)b |
| | Sebacinales | 0.013 (0.007)a | < 0.001b | 0.002 (0.001)ab |
| | Cantharellales | 0.01 (0.004)a | 0.001 (0)b | 0.016 (0.009)a |
| | Pezizales | 0.001 (0)a | 0.009 (0.002)b | 0.005 (0.002)b |
| **Saprotrophs** | Mortierellomycetes | 0.144 (0.014)a | 0.225 (0.015)b | 0.174 (0.013)a |
| | Sordariomycetes | 0.005 (0.001)a | 0.01 (0.002)b | 0.008 (0.002)ab |
| | Wallemiomycetes | 0.003 (0.001)a | 0.011 (0.002)b | 0.006 (0.001)c |
| | Kickxellales | 0.001 (0)a | < 0.001b | 0.001 (0)ab |
| | Basidiobolales | < 0.001a | < 0.002ab | < 0.002b |
| **Pathotrophs** | Polyporales | 0.004 (0.001)a | 0.009 (0.001)b | 0.006 (0.001)c |
| | Spizellomycetales | 0.001 (0.001)a | 0.001 (0.001)ab | < 0.001b |
| | Urocystidales | < 0.001a | 0.006 (0.003)b | 0.002 (0.001)b |
| | Olpidiales | absent | < 0.001b | < 0.001c |
| **Year three** | | | | |
| **EMF** | Agaricales | 0.0293 (0.0092)a | 0.065 (0.0162)b | 0.0445 (0.0101)ab |
| | Mytilinidiales | 0.0059 (0.002)a | 0.0036 (0.0021)ab | 0.002 (0.0011)b |
| | Pezizales | 0.0011 (0.0005)a | 0.0044 (0.0011)b | 0.0063 (0.0024)ab |
| | Gomphales | absent | 0.0018 (0.0014) | absent |
| **Saprotrophs** | Agaricomycetes | 0.0949 (0.0161)a | 0.0696 (0.0127)b | 0.1162 (0.0213)a |
| | Mortierellomycetes | 0.0457 (0.0053)a | 0.0714 (0.0079)b | 0.0673 (0.0058)b |
| | Geoglossomycetes | 0.0145 (0.0039)a | 0.0028 (0.0012)b | 0.0038 (0.0016)b |
| | Umbelopsidomycetes | 0.0023 (0.0007)a | 0.0059 (0.0018)b | 0.0056 (0.0014)b |
| | Tremellomycetes | 0.0013 (0.0005)a | 0.0014 (0.0003)a | 0.002 (0.0005)a |
| | Geminibasidiomycetes | 0.0011 (0.0004)a | 0.0027 (0.0007)b | 0.0032 (0.0013)ab |
| **Pathotrophs** | Rhizophydiales | 0.002 (0.0004)a | 0.0057 (0.0016)b | 0.0046 (0.0017)ab |
| | Spizellomycetales | 0.0013 (0.0008)ab | 0.0001 (0.0001)a | 0.0005 (0.0002)b |
| | Thelebolales | 0.0005 (0.0002)a | 0.0005 (0.0002)a | 0.0013 (0.0004)b |
| | Urocystidales | 0.0004 (0.0002)a | 0.0045 (0.0024)b | 0.0009 (0.0006)ab |
| | Xylariales | 0.0004 (0.0002)a | 0.0003 (0.0002)ab | 0.0001 (0.0001)b |
| | Venturiales | 0.0001 (0)a | 0.0005 (0.0002)b | 0.0016 (0.0011)ab |
| | Eurotiales | absent | 0.0005 (0.0003)b | 0.0003 (0.0002)b |
| | Olpidiales | absent | 0.0001 (0)a | 0.0002 (0.0002)a |

**Figure 1.1**

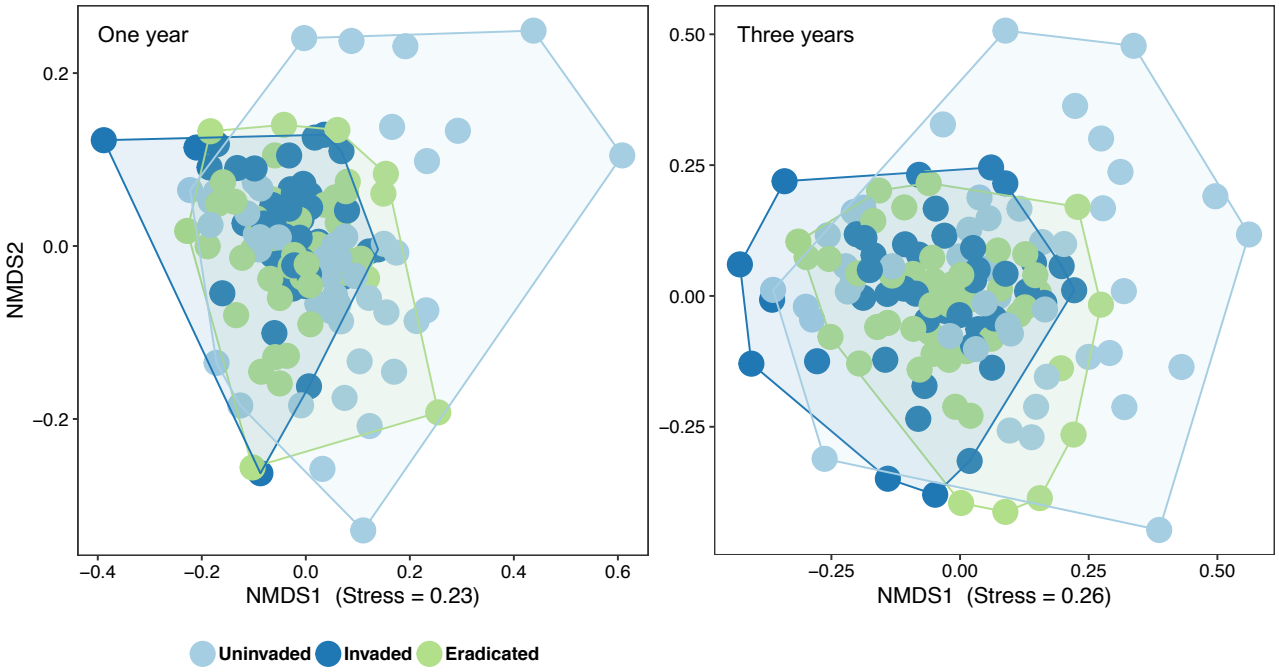


**Figure 1.1.** Fungal community composition one and three years after garlic mustard eradication. Points represent the Bray-Curtis dissimilarity across sites, with the range in dissimilarity across invasion statuses shown using polygons.

**Figure 1.2.**

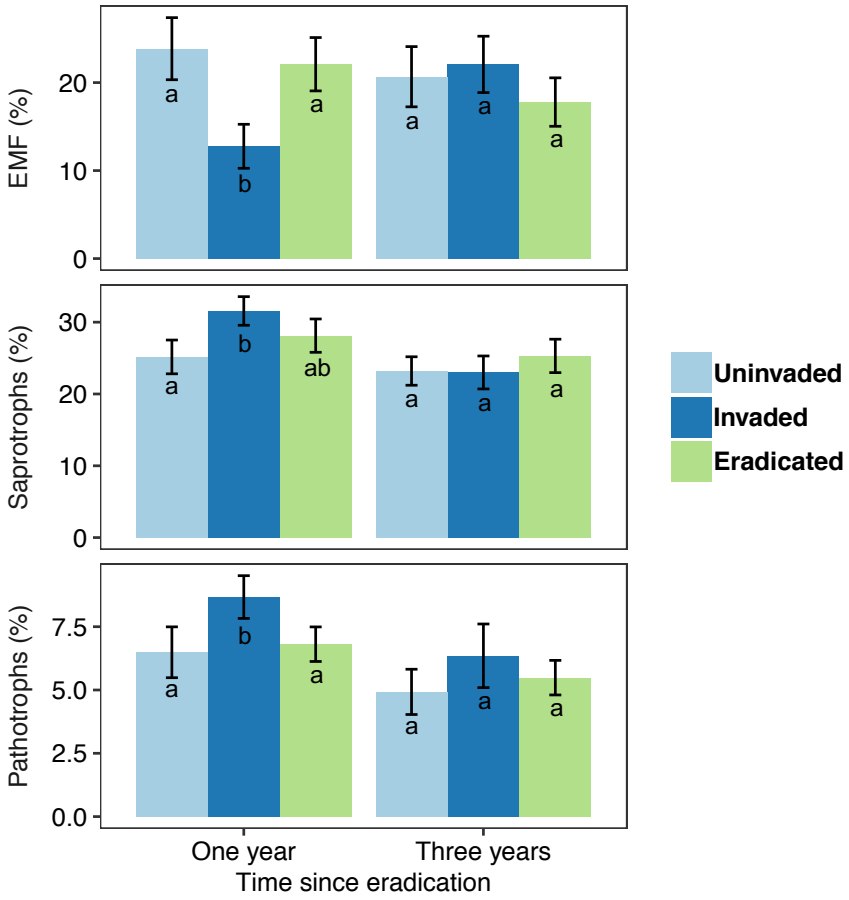


**Figure 1.2.** The relative abundance of fungal functional guilds one and three years after garlic mustard eradication. Bars represent the mean and error bars are the standard error. Different lowercase letters are significantly different.

**Figure 1.3**

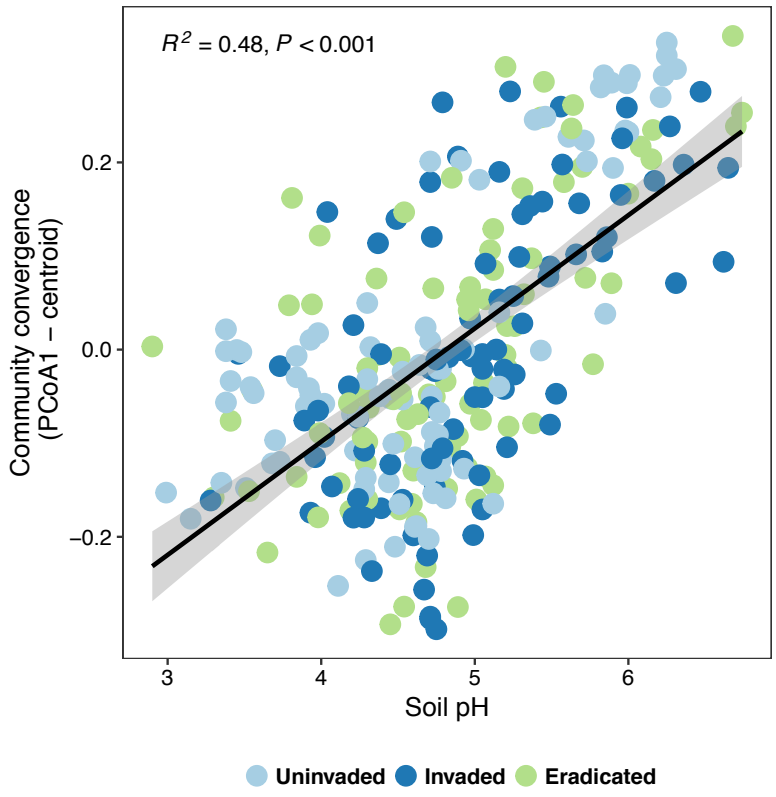


**Figure 1.3**. Community convergence in relation to soil pH across invasion statuses.

**Figure 1.4**

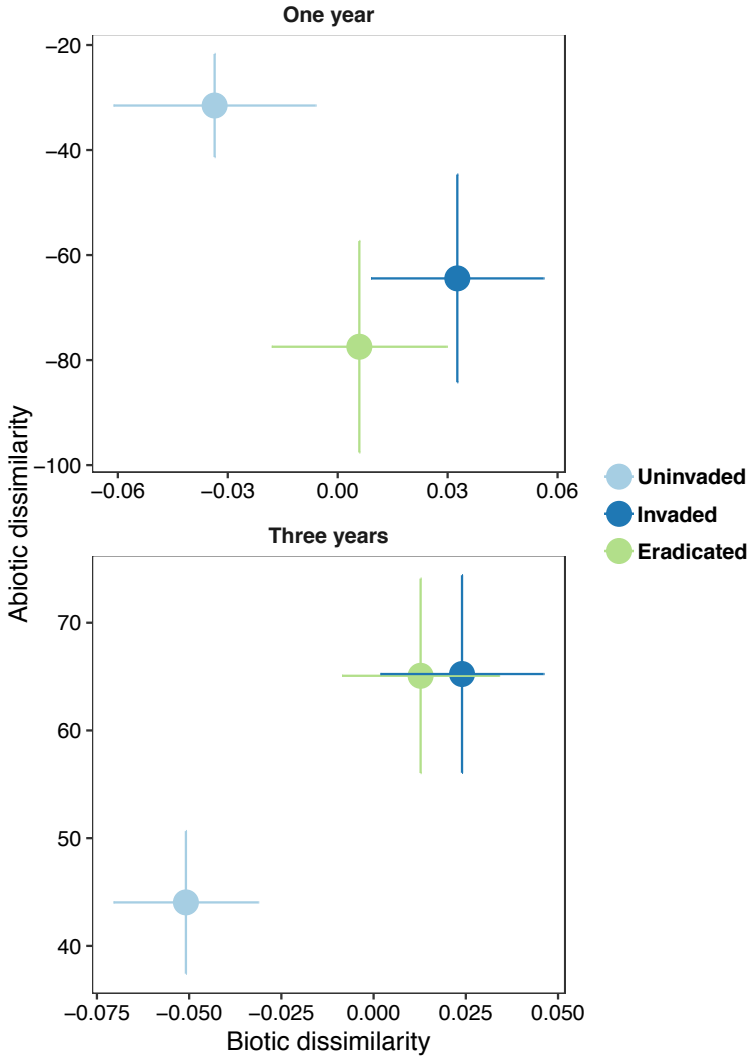


**Figure 1.4**. Overall dissimilarity in the fungal (biotic) and edaphic (abiotic) components of soils one and three years after eradication in the reference uninvaded, invaded, and eradicated plots. Points represent the centroid of fungal community composition (Bray-Curtis) in principle correspondence analysis (axis 1) and edaphic property composition (Euclidean) in principle correspondence analysis (axis 1) and error bars are the standard deviation.

# Chapter 2

## Soil warming amplifies the impacts of biotic invasion on fungal communities

### 1. Abstract

The impacts of plant invasions may be enhanced by climate warming and anthropogenic nitrogen (N) deposition. How fungi respond to this interaction is especially important because fungi are already sensitive to plant invasions under current conditions. Fungi also form mycorrhizas with most native plants and mediate key roles in the carbon cycle as decomposers, so their responses to these interacting stressors could significantly shape native biodiversity and ecosystem function. In this study, we experimentally invaded a long-term soil warming × simulated N deposition experiment with *Alliaria petiolata* (garlic mustard), a non-native plant invasive throughout temperate North America. We measured a suite of soil properties and fungal and arbuscular mycorrhizal fungal biomass and community structure. We found that soil warming was the dominant factor to impact fungal communities, and it interacted with invasion to amplify the impacts of invasion on fungal biomass and community composition. Heat crossed with invasion also gave rise to unique changes in soil properties and processes, which in turn, affected fungal communities via environmental filtering. The importance of environmental filtering in structuring fungal communities increased with warming. Our results show that soil warming can accelerate and amplify the impacts of invasion on soil fungal communities.

## 1. Introduction

Ecosystems around the world are subjected to multiple anthropogenic global change drivers that are expected to worsen over the century and beyond (Galloway *et al*. 2004, Hoegh-Guldberg *et al*. 2018, Seebens *et al*. 2018). This makes it difficult to predict the responses of ecosystems to global change since the combination of multiple stressors can create new impacts that are difficult to test (Aber *et al*. 2001) and are often not studied (Heller and Zavaleta, 2009). Of particular concern for native species diversity (Wilcove *et al*. 1998), the impacts of invasive species could worsen with climate change and nitrogen (N) deposition (Dukes and Mooney, 1999). A greater number of invasive plants can establish under experimental N and water additions (Milchunas and Lauenroth, 1995), and there are frequently higher densities of invasive plants at sites with elevated N availability (Howard *et al*. 2004). However, some invasive plants could become less impactful if global change reduces their overall performance. For example, prolonged growing seasons in North America as a result of climate warming reduces the performance of invasive *Lythrum salicaria* (purple loosestrife) (Colautti and Barrett, 2013). While there is clear evidence that abiotic global change can impact the colonization and performance of invasive plants, we do not understand whether these stressors ameliorate or enhance the impacts of invasive plants on native species diversity.

Once an invasive plant establishes, it is frequently aggressively managed in order to protect native plants from competitive exclusion (Wittenberg and Cock, 2005), which for some plants depends on the soil fungal community response to invasion (Stinson *et al*. 2006, Callaway *et al*. 2008). Some invasive species separate native plants from their mycorrhizal fungal symbionts (Roberts and Anderson, 2001) or increase fungal plant pathogen loadings that intensify disease incidences (Mangla *et al*. 2008). How fungi respond to plant invasions in

combination with climate change and N deposition can therefore help inform adaptive management decisions. Fungi are also important decomposers and they contribute to the formation of stable soil organic matter (Kallenbach *et al*. 2016), making them important components of climate change mitigation. We already know that fungal communities are sensitive to simulated global change stressors when considered individually, including soil warming (Geml *et al*. 2015, Fernandez *et al*. 2017), N deposition (Lilleskov *et al*. 2001, Morrison *et al*. 2016), and plant invasions (Callaway *et al*. 2008), but we do not understand the full complement of these interactions.

In this study we measured fungal taxonomic and community responses to experimental *Alliaria petiolata* (garlic mustard) invasion into a long-term soil warming and simulated N deposition study at the Harvard Forest Long-Term Ecological Research Site (LTER). Fungi have known sensitivities to garlic mustard invasion throughout temperate North America (Rodgers *et al*. 2008a). Garlic mustard is also non-mycorrhizal, and invasion can suppress mycorrhizal fungi (Stinson *et al*. 2006, Wolfe *et al*. 2008), the loss of which has been linked to fewer understory tree seedlings (Stinson *et al*. 2007) and a shift towards increased saprotrophic fungal dominance (Anthony *et al*. 2017). Garlic mustard also grows larger with elevated N (Meekins and McCarthy, 2000) but not warmer temperatures (Anderson and Cipollini, 2013), so there are presumably important interactions among invasion, simulated N deposition, and soil warming on fungal communities. We had two main objectives: (1) to characterize how soil warming, simulated N deposition, and invasion change soil properties (soil moisture, pH, total soil C contents, inorganic N contents, fine root biomass, total fungal biomass, arbuscular mycorrhizal fungal biomass), soil processes (N-mineralization, C-mineralization), and fungal community

structure (general fungal, arbuscular mycorrhizal fungal), and (2) to compare the role of environmental filtering in structuring fungal communities across the global change stressors.

We focus on the role of environmental filtering because environmental conditions frequently shift with global change drivers, and this could impact fungal communities (Kivlin *et al*. 2014). Fungal lineages tolerate differential temperatures (Miyamoto *et al*. 2018), pH ranges (Glassman *et al*. 2017), nutrient availabilities (Bödecker *et al*. 2014), C:N ratios (Thomson *et al*. 2015), and a host of other environmental conditions that can select for particular taxa via environmental filtering (Kivlin *et al*. 2014). Fungi are also sensitive to plant invasions because invasion can alter soil conditions that resident fungi adapted to inhabit (Anthony *et al*. 2017). The role of environmental filtering is also balanced beside stochastic processes related to dispersal and neutrality (see Powell *et al*. 2015, Powell and Bennet, 2016). Disturbances like wildfire (Ferrenberg *et al*. 2013) and cattle grazing (Caruso et a. 2012) can reduce the importance of environmental filtering, consequently making community assembly more stochastic. It is therefore possible that changes in environmental filtering, in addition to the relative importance of environmental filtering versus stochastic processes, drives fungal responses to more than one global change stressor.

## 2. Materials and Methods

### *2. Site description and experimental design*

This work was conducted at the Soil Warming × Nitrogen Addition (SWaN) experiment located at the Harvard Forest LTER in Petersham, MA (42°29′15″N 72°11′15″W). SWaN was established in an even-aged, mixed stand with a canopy of red and black oak (*Quercus rubra, Q. velutina*), red and striped maple (*Acer rubrum, A. pensylvanicum*), American beech (*Fagus gradifolia*), white birch (*Betula papyrifera*), and understory sprouts of American chestnut

(*Castanea denata*). Annual temperature at Harvard Forest is 8.3°C and annual precipitation is 1,247 mm (c. 2002-2018; Boose, 2018). This experiment was initiated in 2006 to study interactions between soil warming and simulated N deposition (see Contosta *et al*. 2011). Warming is achieved using buried heating cables (+5°C ambient temperature), simulated N deposition plots are fertilized with aqueous $NH_4$-$NO_3$ at equal doses throughout May-October (+5 g N $m^2$ $year^{-1}$), and warming × nitrogen plots experience both treatments. In April 2015, we established uninvaded and invaded 1 $m^2$ subplots within five of the six replicate treatment plot, and experimentally introduced *A. petiolata* (garlic mustard) at densities typical to the region (a detailed description of which can be found in Wheeler *et al*. 2017). The number of garlic mustard plants in each plot was maintained throughout the invasion to be similar across treatments. The full experiment has three factors (nitrogen warming, invasion) with eight different levels (control, warming, nitrogen, invasion, warming × nitrogen, warming × invasion, nitrogen × invasion, and warming × nitrogen × invasion) and 40 plots (8 treatment levels × 5 replicates).

*2. Soil Sampling and Processing*

Soil samples were collected in July 2016 by removing 100 $cm^2$ rectangles from the organic horizon to the depth of the mineral soil and then 5 cm of mineral soil using a soil corer (5 cm width × 10 cm depth). Two samples were taken from each plot and homogenized together but kept separate based on depth increment. Homogenized samples were stored on frozen ice packs in the field and immediately placed at 4°C within 12 hours of sampling. Samples were sieved (<4 mm) within 24 hours of sampling and roots, rocks, and organic debris ≥4 mm were removed. We sieved soils through a 4 mm sieve in order to keep fine roots (<2 mm). Subsamples (~2 g) for molecular analysis and lipid analysis (~10 g) were taken from the sieved material and stored at -80°C and -20°C until processing, respectively

*2. Edaphic Analyses*

Soil pH was measured on soil slurries made from dried soil and deionized water (10 g soil : 20 mL deionized water). Total soil C and N was measured on dried, finely ground soils using an elemental analyzer (Perkin Elmer 2400 Series II CHN elemental analyzer, Waltham, MA). Total soil C was calculated on a volumetric basis using bulk density measurements previously made at the site (unpublished data). Soil inorganic N concentrations were determined on 2M KCl extracts (10 g soil : 40 mL KCl) using a colorimetric approach (Braman and Hendrix, 1989). Net N mineralization was also measured after a seven-day laboratory incubation. In a separate incubation, we analyzed C mineralization using a ten-day laboratory incubation. We measured soil respiration daily from Mason jars with sealed septa containing 10 g of field moist soil and calculated average respiration rates as a proxy for C lability. Fine root biomass was estimated by picking fine roots (≤2 mm) from 100 g of fresh soil, and we calculated fine root stocks on a volumetric basis using soil bulk density.

Microbial biomass was assessed via phospholipid and neutral lipid fatty acid analysis (P/N-LFA). For total fungi, we used PLFA analysis, and for AMF we used NLFA analysis consistent with Olsson *et al*. (1995). In short, lipids were extracted from freeze-dried soil (1 g) using phosphate buffer, chloroform, and methanol (0.8:1:2; v:v:v). The polar (phospholipids) and neutral lipids were isolated separately using silicic acid chromatography and methylated using 0.2M methanolic potassium hydroxide (1 mL) at 60°C for 30 minutes to form fatty acid methyl esters (FAMES) that were quantified on a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (Agilent Technologies, Santa Clara, CA). We compared FAME peaks against a standard library of FAMES specific to fungi (18:2ω6, 9c, 18:1ω9c) and AMF

(16:1ω5c). Standards for each marker were used convert peak area concentrations into nmol PLFA/NLFA $g^{-1}$ dry soil.

*2. Fungal community characterization*

Fungal and AMF community structure was characterized using ITS2 and 18S metabarcoding on the Illumina MiSeq platform, respectively. DNA was extracted from soil (0.25 g) using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). The ITS2 region was amplified using the fungal specific primer pair *f*ITS7 (Ihrmark *et al*. 2012) and ITS4 (White *et al*. 1990). Primers currently developed to study fungi poorly cover the Glomeromycotina and therefore the 18S region was also amplified using the Glomeromycotina (AMF) specific primer pair NS31 (Simon *et al*. 1992) and AML2 (Lee *et al*. 2008). In the results section, we refer to fungi identified by the ITS2 region as, 'fungi', and AMF identified by the 18S region as, 'AMF'. PCR primers contained the Illumina adaptor sequence, an 8 bp pad sequence, a 2 bp linker sequence, and were dual indexed to include two unique 8 bp sequences (see custom PCR primer constructs, Supplementary Table 2.1). PCR reactions were performed in triplicate for each sample in 25 µL reactions with the following reagents: PCR Grade $H_2O$ (13 µL), Phusion® High-Fidelity PCR Master Mix with HF Buffer (10 µL; New England BioLabs Inc, Ipswitch, MA), 10 µM forward primer (0.5 µL), 10 µM reverse primer (0.5 µL), and template DNA (1 µL). Thermocycler conditions followed that of Anthony *et al.* (2017). PCR products were cleaned using the AxyPrep MAG PCR Clean-up kit (Corning, Tewksbury, MA). Final PCR products were inspected on an agarose gel and DNA concentration was measured by fluorometry on a Qubit® 3.0 Fluorometer (Life Technologies, Grand Island, NY). Equimolar libraries of the ITS2 and 18S samples were sequenced on an Illumina MiSeq v2 (2 x 250 bp) run and two MiSeq v3 (2 x 300 bp) runs at the Center for Genomics and Bioinformatics at Indiana State University, Bloomington, IN,

respectively. Sequences are available in the NCBI database under the BioProject PRJNA522440 for ITS2 sequences and PRJNA522442 for 18S sequences.

All sequences were passed through a series of quality control measures. We first removed Illumina adapter and PCR primer sequences, reads <100 bp, and low-quality bases and reads (Phred scores < 2) using Trimmomatic (v0.32; Bolger *et al*. 2014). We then merged forward and reverse reads using the join_paired_ends.py function in QIIME (Caporaso *et al*. 2010). The ITS2 reads were merged at a 20 bp overlap allowing 5% mismatch. The 18S reads were merged at a 10 bp overlap allowing 10% mismatch. Merged ITS2 sequences were then passed through ITSx (Bengtsson-Palme *et al*. 2013) to isolate the ITS2 region from flanking 18S and 5.8S regions. A total of 60% and 62% of initial paired end sequences were retained for the ITS2 and 18S datasets, respectively, after quality control (see Supplementary Table 2 for a step-by-step list of sequence retention). We used USEARCH (v8) to create OTU tables (Edgar, 2010). Sequences were dereplicated (-fastx_uniques), sorted by size with singletons removed (-sortbysize), and clustered at 97% sequences similarity with chimera removal (-cluster_otus). We assigned taxonomy to ITS2 OTUs using the UNITE reference database (version 7; January 2016 release) and the assign_taxonomy.py function in QIIME. 18S OTUs were blasted against the MARJAAM database (Öpik *et al*. 2010). OTUs without a match to fungi in the UNITE or MARJAAM databases were then blasted against the entire NCBI *nr* database, and OTUs assigned to non-fungal organisms for ITS2 data or non-Glomeromycotina fungi for the 18S sequences were removed from subsequent analyses. Fungi from the ITS2 dataset were assigned guild annotations using FUNGuild and all "probable" or higher designations were included (Nguyen *et al*. 2016). Information on the number of OTUs and proportion of sequences assigned taxonomy and guild annotations is in Supplementary Table 2.2.

*2. Variation in soil variables, processes, and fungal biomass*

All statistical analyses were conducted in R (R Development Core Team, 2008), with significance for all tests set at $P \leq 0.05$. Models were set-up with three factors: abiotic global change treatment (warming, nitrogen, and warming × nitrogen), invasion, and soil horizon. We kept the abiotic global change treatment and invasion as separate factors to reduce the number of factor levels, and specifically tease apart whether impacts of invasion shifted in the context of the abiotic treatments. We therefore looked at all two- and three-way interactions to understand how treatment and invasion interacted and varied across soil horizons. We used linear mixed effects models and the nlme package in R (Pinheiro *et al*. 2007). Replicate was used as a random variable in all models. Variables were not transformed before analyses. We created beyond optimal models that included statements for autocorrelation and unequal variance across experimental levels when this improved model fit based on significantly reduced AIC scores. For all models, we confirmed that residuals were normally distributed using Shapiro-Wilk tests of normality and qqnorm plots. We performed multiple comparisons using Dunnet's approach to determine where a treatment value deviated from control plots using *t*-tests with unequal variance (heteroscedastic).

*2. Variation in fungal community composition*

Variation in fungal community composition across the abiotic global change treatments, invasion, and horizon was assessed on fungal relative abundances converted to Bray-Curtis dissimilarities using the vegdist function in vegan (Oksanen *et al*. 2007). Bray-Curtis dissimilarities were then assessed for significant dissimilarity using PERMANOVA on the whole dataset, and then for pair-wise variation across significant treatment levels using pair-wise PERMANOVA multiple comparisons. Fungal community composition was visualized on

Wisconsin-square root, double-transformed relative abundances in order to equalize importance of OTUs and Bray-Curtis dissimilarities using non-metric multidimensional scaling (NMDS). We also analyzed variation in coefficients of determination based on joint species distribution modeling (see *Analysis of environmental filtering and fungal realized niches*) based on Euclidean distances and principle coordinates analysis (PCoA) using the ape package (Paradis *et al*. 2004).

*2. Analysis of environmental filtering and fungal realized niches*

We used a Bayesian hierarchical joint species distribution modeling (JSDM) approach called Hierarchical Modeling of Species Communities (HMSC; Ovaskainen *et al*. 2017) to model the influence of soil properties (*i.e.,* soil horizon, soil moisture, soil pH, inorganic N contents, total soil C) and processes (*i.e.,* N mineralization and C mineralization; hereafter: C lability) on taxonomic relative abundances (*i.e.* realized niches). We only modeled taxa that were found across ≥40% of the plots as this degree of presence was necessary for making accurate model predictions. This threshold included 57 fungal (ITS2) and 48 AMF (18S) taxa which we modeled as separate groups. We only included predictor variables that were not highly multicollinear ($r^2 < 0.5$). We created separate models for each treatment level in order to compare the influence of soil parameters on fungal relative abundances and the amount of residual variation across treatment levels. We only created models for control plots and treatment levels where fungal community composition was significantly different from control plots. Every model included a latent, random variable that captures variation unexplained by predictor variables. The random variable is indicative of stochastic processes (Ovaskainen *et al*. 2017) and/or unmeasured soil variables that are not autocorrelated with the primary soil parameters included as predictor variables. Our primary outputs included the amount of residual variation as

an indication of environmental filtering effects versus stochastic processes and the impact of different soil parameters on fungal relative abundances.

HMSC was implemented in R using the *HMSC* package (Ovaskainen *et al*. 2017). Models were created using an over-dispersed Poisson distribution modelling approach (family=overPoisson). For the ITS2 data, models were run for 1,000,000 iterations, with 100,000 burn-ins (initial throw-out iterations) and thinned by 10,000 to reduce autocorrelation. For the 18S data, there were 100,000 iterations, 10,000 burn-ins, and we thinned by 1,000. These model parameters were selected after different iterations to make sure models were well-mixed (Supplementary Figures 2.1 & 2.2). We performed variance partitioning using the *varipart* function in the HMSC package to measure the relative influence of predictor variables on relative abundance. The variance partitioning output includes an estimated coefficient of determination for each taxon and predictor variable (including the random effect), and this matrix was analyzed as a Euclidean distance to calculate each taxon's overall 'realized niche' (*sensu* Ovaskainen *et al*. 2017). We used PERMANOVA to determine whether realized niches of species differed across the treatments and PERMDISP to measure whether the heterogeneity of realized niches increased or decreased across treatments. If there is more or less heterogeneity in realized niches then this reflects increased and decreased niche partioning, respectively. These results were visualized using PCoA with the loading values for individual soil parameters calculated using the *envfit* function in vegan.

## 2. Results

### *2. Soil properties, processes, and fungal biomass*

Overall, soil properties, processes, and fungal biomass pools were most altered by warming, especially warming × invasion (Table 2.1). In contrast, invasion alone had no impact on any soil

parameters. Total soil C was negatively affected by warming, warming × invasion, warming × nitrogen × invasion, and to a lesser extent nitrogen additions alone. Changes in total soil C were only significant for the organic horizon, except for warmed plots where mineral soil C content was also reduced. C mineralization (hereafter referred to as C lability), fine root biomass, and fungal and AMF biomass were affected by warming × invasion but not warming or invasion alone. C lability was lower, fine root biomass increased, and fungal and AMF biomass decreased in the warming × invasion compared to control plots. A number of soil properties and processes were not significantly altered by any of the treatments, including soil pH, inorganic N contents, and N mineralization (Supplementary Table 2.3).

*2. Fungal community composition*

Fungal (ITS2) community composition was significantly affected by the abiotic global change treatments (PERMANOVA: $P = 0.002$; Supplementary Table 2.4) but not by biotic invasion alone ($P = 0.5$). The effects of the abiotic global change treatments on community composition further varied between invasion statuses, giving rise to a significant abiotic global change treatment × invasion interaction ($P = 0.04$). None of these effects interacted with soil horizon. Across the abiotic global change × invasion interaction, community composition was different in the warming, warming × invasion, and warming × nitrogen × invasion plots compared to control plot (Figure 2.1a). Communities in the warming and warming × nitrogen × invasion plots were similar but distinct from communities in the warming × invasion plots. AMF (18S) community composition was also significantly affected by the abiotic global change treatments ($P = 0.006$), but also by biotic invasion ($P = 0.03$) and their interaction ($P = 0.03$). None of these effects interacted with soil horizon (Supplementary Table 2.4). AMF community composition in the warming and warming × invasion plots were distinct from communities in

control plots and from each other (Figure 2.1b). While AMF community composition was distinct across all uninvaded compared to invaded plots, in the control plots alone, there was no impact of invasion.

*2. Fungal relative abundances and correlations to soil properties and processes*

Overly dominant fungi differentiated fungal communities in warmed plots compared to control plots, and these dominant taxa were especially sensitive to invasion (Figure 2.2). The ectomycorrhizal taxon, *Russula sp. 11*, was dominant in the warmed (28%) compared to control (2%) plots ($P = 0.04$) and was nearly absent from the warming × invasion (<1%) plots ($P = 0.03$; Figure 2a). Loss of *Russula sp. 11* in the warming × invasion plots significantly reduced the total relative abundance of EMF ($P = 0.02$; Supplementary Figure 2.3). Similarly, a single AMF that could not be identified beyond the genus level (*Glomus species* 3) was highly dominant in the warmed (46%) compared to control (23%) plots ($P = 0.03$), and its relative abundance was lowest in the warming × invasion (13%) plots ($P = 0.005$; Figure 2.2b). The dominant taxa in warming plots were especially sensitive to invasion while other, less dominant taxa increased in relative abundance in the warming × invasion plots. Three of the four most abundant general saprotrophs, *Mortierellaceae sp. 7, Umbelopsis sp. 41,* and *Umbelopsis sp. 210*, were at highest relative abundances in the warming × invasion plots, raising the overall relative abundance of general saprotrophs ($P = 0.05$; Supplementary Figure 2.3). For AMF, the relative abundance of *Glomus sp. 1* was highest in the warming × invasion (42%) compared to control (20%) plots ($P = 0.03$), and to a lesser extent warming alone (22%; $P = 0.06$). The second most common AMF genus, *Scutellospora,* was not affected by warming × invasion (6%) compared to control (12%) plots ($P = 0.13$), but their relative abundance was significantly lower in the warmed (3%) plots compared to control plots ($P = 0.03$).

The relative abundances of common fungi (taxa present in >40% of the plots) were largely accounted for the soil parameters we measured, but the amount of variation explained differed across the global change treatments (from 60-98%; Figure 2.2). Soil parameters accounted for less variation in fungal relative abundances in control plots compared to the treatment plots and their interactions ($P_{\text{Fungi (ITS2)}} < 0.0001$; $P_{\text{AMF(18S)}} = 0.02$). In other words, variation in fungal relative abundances was explained by soil parameters to a greater extent in treatment plots compared to control plots. Taxa with high relative abundances also had more of their variation in relative abundances explained by soil parameters than taxa with lower relative abundances. Taxa whose relative abundance decreased or increased in the treatment groups also had reduced and increased variation explained by soil variables, respectively. For example, the total relative abundance of *Russula cyanoxantha* + *Russula sp. 11* was highest in the warming × nitrogen × invasion plots followed by the warming and then control and warming × invasion plots. The relative abundance of these taxa was significantly correlated to total soil C in the warming × nitrogen × invasion plots ($R^2 = 0.82$, $P = 0.0003$; Figure 2.3), but this correlation was not significant in the warming only plots ($R^2 = 0.32$, $P = 0.08$), and it was absent in the control and warming × invasion plots.

Fungal taxonomic 'realized niches', measured as the multivariate influence of all modeled soil parameters on taxonomic relative abundances, shifted in the treatment compared to control plots. Both Fungal (ITS2) and AMF (18S) realized niches varied across the treatment plots ($\text{PERMANOVA}_{\text{ITS2}}$: $R^2 = 0.47$, $P = 0.001$, Figure 2.4a; $\text{PERMANOVA}_{\text{18s}}$: $R^2 = 0.28$, $P = 0.001$, Figure 4b), but realized niche heterogeneity did not ($\text{PERMDISP}_{\text{ITS2}}$: $F = 0.09$, $P = 0.96$; $\text{PERMDISP}_{\text{18s}}$: $F = 1.63$, $P = 0.2$). Soil moisture and the random variable were most indicative of fungal (ITS2) realized niches in the control plots. Soil pH and total soil C were most indicative in

warming and warming × invasion plots. C lability was the only variable strongly indicative in the warming × nitrogen × invasion plots and was negatively correlated to fungi in the warmed plots. For AMF, C lability and soil pH were most indicative of realized niches in control plots, while many soil variables, including soil moisture, inorganic N concentrations, soil depth, and total soil C were most indicative in the warmed plots. N mineralization was most indicative of realized niches in the warming × invasion plots. The coefficient of determination for every soil variable for fungi (ITS2) and AMF (18S) are shown individually in Supplementary Figure 2.4. Overall, the same fungi in control plots were differentially correlated to soil parameters in the treatment plots, indicative of gains and losses in fungal niches.

## 2. Discussion

The impacts of invasive plants may worsen with global change, but few studies have measured the effects of invasion in the context of other global change stressors (Wheeler *et al*. 2017). Simulation models largely predict the spread of invasive species into new ranges in temperate North America in response to climate warming (*i.e.* Peterson *et al*. 2008, Bradley *et al*. 2010), suggesting the need to test whether impacts of invasive species could be alleviated or enhanced in combination with other global change stressors. In this study, we measured the response of fungal communities and a suite of soil parameters to experimental garlic mustard invasion in the context of a long-term soil warming × simulated N deposition study. We show for the first time that warming can amplify the impacts of an invasive plant on soil properties and general fungal and AMF communities. A small number of overly dominant fungi were selected for by warming, and these taxa were more sensitive to invasion than fungi in control plots. Fungi in heated plots were also more strongly impacted by environmental filtering relative to those in control plots, indicating enhanced niche-based assembly as an adaption to heat. As a result, the

added invasion treatment disrupted species niches more with heat than under ambient conditions, giving rise to steeper impacts of invasion in the context of warming.

*2. Warming gave rise to unique impacts of invasion on soil properties, processes, and fungal biomass*

Soil parameters were altered by the global change treatments in combination with invasion in unique ways from the individual treatments. Invasion alone had no impact on soil properties, even though it is known to affect soil pH, inorganic N availability, and soil C contents in long-term, established invasions (Rodgers *et al*. 2008b, Anthony *et al*. 2017). Since the experimental invasion in this study was only sustained for a single life cycle of garlic mustard (two years), it was apparently insufficient to impact soils in the ambient plots. It is therefore especially interesting that invasion reduced C mineralization (a proxy for C lability), fungal biomass, and AMF biomass in concert with soil warming when none of these parameters were significantly decreased by invasion or warming alone. Reduced C lability could be related to lower fungal biomass, especially since there was also reduced total soil C content in this treatment. Reduced access to soil C can inhibit fungal growth and reduce biomass (Paustian and Schnürer, 1987). Total soil C content, but not C lability, was also lower in the individual global change plots. Total soil C loss is a well-known outcome of long-term soil warming (Crowther *et al*. 2016), and there have been sustained increases in soil respiration over the course of this experiment in the warmed plots, and to a lesser extent those where nitrogen is added (Contosta *et al*. 2011). The already reduced soil C pool in warmed plots may have created a cascade of effects when we invaded the plots with garlic mustard, reducing C lability and fungal biomass.

There were also more fine roots in the global change plots that we invaded with garlic mustard. Some of these roots were seemingly from the garlic mustard. Since garlic mustard roots

contain high levels of glucosinolates (Vaughn and Berhow, 1999), secondary plant compounds that suppress fungi (Wolfe *et al*. 2008, Cantor *et al*. 2011), this could help explain loss of fungal biomass in the warmed plots. However, increased fine roots were likely also produced by native trees in response to reduced mycorrhizal biomass. An earlier analysis of the red maple (*Acer rubrum*) tree seedlings in this same experiment showed that warming × invasion reduced AMF colonization (Wheeler *et al*. 2017). The loss of AMF could cause native plants to compensate by producing more roots in order to access soil nutrients typically scavenged by AMF (Smith and Read, 2010). We also hypothesize that EMF biomass was reduced since their relative abundance was lowered by invasion in combination with warming (Supplementary Figure 2.3) and because total fungal biomass was reduced (Table 2.1). This could cause ectomycorrhizal trees to produce more fine roots for the same reason as AMF associated vegetation since trees with both mycorrhizal types are common at the experiment.

*2. Heat is the dominant stressor to affect fungal community composition, but it gives rise to different communities when crossed with invasion*

We clearly show that soil fungal communities were sensitive to warming in a temperate forest understory. Both general fungal and AMF communities shifted in response to soil warming. Changes in the fungal community may be related to aforementioned losses in total soil C independent of invasion, especially since soil C loss was greatest in the treatments where fungal communities also turned over. Earlier work at this experiment found that soil organic matter in the warmed plots has more oxidized lignin and other plant derived compounds that fungi primarily decompose via oxidative enzymes (Pisani *et al*. 2015). Since different fungal species possess varying capacities for decomposition of these substrates (Kohler *et al*. 2017, Tang *et al*. 2016), and fungi are likely the main decomposers in forests soils (Schneider *et al*.

2012), turnover in the fungal community is presumably related to total soil C loss, though experimental work is needed to understand any of these mechanisms.

Soil warming selected for fungal species that were overly dominant but less resistant to invasion than fungi in ambient plots. Neither general fungal nor AMF communities were sensitive to invasion in the control plots, likely because invasion was only sustained for one biennial life cycle of garlic mustard. Earlier work on garlic mustard invasions has found that the initial stages of invasion do not impact fungal communities like established invasions (Lankau, 2011), so the more immediate impacts of invasion in the warmed plots suggests that warming accelerated the impacts of invasion. To that end, invasion in the warmed plots generated two results consistent with long-term field observations of garlic mustard invasions (*sensu* Anthony *et al*. 2017, 2019). First, it reduced the total relative abundance of EMF, which were the dominant functional group (Supplementary Figure 2.3). We identified the genus *Russula* to be most dominant and especially responsible for loss of EMF and turnover in the fungal community, which is consistent with other global change studies in deciduous northeastern forests. Notably, *Russula vinaceae* drives sensitivities of fungal communities to multiple decades of simulated N-deposition at an experiment nearby the one where this work was conducted (Morrison *et al*. 2016). *Russula* are also particularly sensitive to established garlic mustard invasions across southern New England (Anthony *et al*. 2017) and are frequently the dominant EMF on native tree roots in uninvaded areas of forest but not invaded areas (Castellano and Gorchov, 2012). Second, invasion increased the total relative abundance of general saprotrophic fungi, which were the second most dominant functional group (Supplementary Figure 2.3). In particular, the relative abundance of Mucoromycotina (*e.g.,* Mortierellomycetes, *Umbelopsis*)

increased, and these fungi are at particularly high relative abundances in long-term field invasions (Anthony *et al*. 2017, 2019).

The other mycorrhizal group common in these forests are AMF, which have well-known sensitivities to garlic mustard invasion (Barto *et al*. 2011, Lankau *et al*. 2014). Members of the genus, *Glomus,* were most common, and *Glomus species* 3 was highly dominant in the warmed plots but sensitive to invasion. Conversely, *Glomus sp. 1*, which was also more abundant in the warmed plots increased even more with invasion. These two *Glomus* taxa represent 45-75% of the community, and they both positively responded to warming but in opposite ways to invasion, making communities in the warmed plots especially vulnerable to invasion compared to plots where these taxa were less dominant. The other genus of AMF that were common in these soils, *Scutellospora*, were sensitive to warming but this sensitivity was ameliorated in the invaded plots. *Scutellospora* are known to produce extensive fungal biomass in soil, making them competitive against other AMF (Chagnon *et al*. 2013), but a higher C cost to host plants (Lerat *et al* 2003). In warmed plots, the cost to host plants may not be worth allocating C resources to *Scutellospora* if soil resources are already more available due to increased decomposition (see Contosta *et al*. 2011). Overall, our results show that soil warming can select for arbuscular mycorrhizal and ectomycorrhizal taxa that are overly dominant but sensitive to invasion.

*2. Taxa that positively responded to warming and invasion inhabited suitable realized niches in the treatment plots*

Since most fungi have not been cultured or assigned particular traits, biodiversity theory has been central to inferring why relative abundances differ across environmental conditions (Valyi *et al*. 2016). Niche theory is commonly used to make sense of changes in fungal communities based on environmental filtering (*e.g.,* Lilleskov *et al*. 2001, Kivlin *et al*. 2014,

Morrison *et al*. 2016, Anthony et a. 2017, Glassman *et al*. 2017), Below, we discuss two major findings from our joint species distribution analysis that provide novel insight into fungal species realized niches and how they shift with global change stressors.

First, the proportionally abundant taxa had nearly all of their relative abundances predicted by soil parameters. We interpret this to mean that these taxa are tolerant of soil conditions and are using soil resources (*i.e.,* achieving a realized niche). Species abundance distributions show that plants and animals achieve high abundances because of certain environmental conditions (Whittaker, 1956, Brown, 1984), and that as environmental conditions shift abundance proportionally decreases. Species distribution models mathematically account for these relationships (Meier *et al*. 2010). For soil fungal communities, extreme hyper-dominance by just a few taxa is typical (Dumbrell *et al*. 2010). Since we do not know the fundamental niches of most fungal species, our study suggests that the most dominant fungi, like plants and animals, shift relative abundances in relation to their realized niches. Our results also suggest that hyper-dominance is due to fungi achieving their realized niche with comparatively less dominant taxa failing to inhabit suitable realized niches. It is also important to note that we only modeled fungi present in >40% of the plots. There is less reason to expect rare species to assemble via similar niche processes. Rarity frequently arises from reasons unrelated to the environment, including stochastic processes like recruitment limitations (Hurtt and Pacala, 1995) as well conserved features that make some microbes uncompetitive and chronically rare (Jousset *et al*. 2017).

Second, the overall amount of variation in relative abundances explained by measured soil parameters, a proxy for environmental filtering (Kivlin *et al*. 2014), increased with the global change treatments. There was a greater amount of variation attributed to the random

variable for species relative abundances in the control plots compared to treatment plots. Since the random variable accounts for variation generated by unmeasured environmental properties and/or stochastic processes (Ovaskainen *et al*. 2017), the treatments either decreased the importance of stochastic processes (*i.e.* increased the importance of niche processes like environmental filtering) or decreased the importance of unmeasured, independent soil parameters compared to control plots. The latter seems unlikely since unmeasured soil parameters, like phosphorus availability, would be autocorrelated to measured soil variables, like soil pH and total soil C content (Devau *et al*. 2009). There were also no significant correlations between the taxa we modelled and the molecular constituents of soil organic matter, as assessed by nuclear magnetic resonance (unpublished results). Other disturbances like fire (Ferrenberg *et al*. 2013) and cattle grazing (Caruso *et al*. 2012) can decrease the importance of niche assembly and environmental filtering. Here, we show that soil warming appears to have the opposite effect, increasing the importance of niche assembly and environmental filtering. Since fungi in the warmed plots were more strongly affected by environmental filtering than fungi in control plots, changes in soil parameters because of garlic mustard likely disrupted species niches to a greater extent under warming than ambient conditions. If communities are organized more around niche processes then they may be more at risk to global change compared to communities with greater stochastic assembly.

*2. Fungal realized niches shifted alongside fungal community composition but the extent of niche partioning did not*

Soil properties that predicted the relative abundance of taxa in control plots shifted with the global change treatments. Most notably, the two dominant fungi, *Russula cyanoxantha* and *Russula sp. 11*, were not correlated to total soil C contents in control or warming × invasion plots

(Figure 3), but they were weakly and positively correlated to total soil C in the warming plots and strongly correlated in the warming × nitrogen × invasion plots. It is common for soil property correlations to be stronger with certain community types (Kivlin *et al*. 2014). For example, AMF communities are more strongly correlated to total soil C in forests than grasslands (Davison *et al*. 2015) and to soil N content in unfertilized soils compared to fertilized soils (van Diepen *et al*. 2013). It is less well known how correlations with soil properties change among the same group of taxa, which can represent unique changes in biotic interactions like niche partioning (Ovaskainen *et al*. 2017). We found that fungal relative abundances in the warming plots were more strongly correlated to most soil variables compared to control plots, as discussed earlier with increased overall importance of environmental filtering. Notably, C lability was negatively correlated to the relative abundances of fungi in the warming treatments, where C lability was also reduced. Heterogeneity among fungal realized niches did not decrease in the treatment plots. In other words, redundancy in fungal realized niches did not change, indicating no change in the extent of niche overlap.

In conclusion, this study shows that soil warming, but not simulated N deposition, increased vulnerability of fungi and AMF to biotic invasion. Our results indicate that the impacts of garlic mustard invasion could be accelerated and amplified with climate warming. We also show that the underlying environmental properties that structure fungi and AMF communities shifted with soil warming, and that overall environmental filtering processes were more important under soil warming than under ambient conditions. Increased importance of environmental filtering due to soil warming likely predisposed the fungal community to being altered by invasion by disrupting species realized niches. It suggests that communities assembled primarily around environmental filtering are more vulnerable to global change than communities

assembled more by stochastic processes. Our results can be interpreted as an early warning to prepare for potentially steeper impacts of invasion in the face of climate change. This is especially important as recent work suggests that many non-native plant invasions in this area will increase as the climate warms (Bradley *et al*. 2010) and if the impacts of invasion sharpen, as they did with garlic mustard, then climate warming could exacerbate changes to native diversity and ecosystem function.

**Table 2.1.** Soil properties, processes, and fungal biomass*. Values represent the mean, with standard errors in parentheses. Values in bold are significantly different from the control.

| Soil horizon | Treatment | Soil moisture (g $H_2O$ $g^{-1}$ soil) | Soil pH | Total soil C (g C $m^{-2}$) | Inorganic N (μg N $g^{-1}$ dry soil) | C min (mg $CO_2$ $g^{-1}$ dry soil $d^{-1}$) | N min (μg N $g^{-1}$ dry soil $d^{-1}$) | Fine root biomass (mg dry roots $m^2$) | Fungal biomass (nmol PLFA $g^{-1}$ dry soil) | AMF biomass (nmol NLFA $g^{-1}$ dry soil) |
|---|---|---|---|---|---|---|---|---|---|---|
| Organic | Control | 0.51 (0.12) | 4.0 (0.2) | 3533 (639) | 16.5 (6.6) | 131 (31.4) | 5 (1.2) | 27 (7) | 47 (6) | 227 (26) |
| | Warming | 0.46 (0.11) | 4.2 (0.2) | **1405 (364)** | 25.8 (7.4) | 107 (62) | 5.1 (2) | 24 (1) | 55 (15) | 229 (45) |
| | Nitrogen | 0.49 (0.11) | 3.9 (0.1) | **2107 (343)** | 28.1 (8.4) | 128 (33) | 5.4 (2.5) | 23 (3) | 59 (8) | 285 (63) |
| | Invasion | 0.54 (0.14) | 4.0 (0.1) | 3319 (875) | 29.5 (8.8) | 118 (43) | 4.7 (1) | 30 (5) | 53 (9) | 215 (47) |
| | Warming × Nitrogen | 0.4 (0.12) | 4.2 (0.1) | 2274 (609) | 23 (2.8) | 79 (39) | 3.4 (1.4) | 41 (17) | 47 (8) | 257 (93) |
| | Warming × Invasion | 0.37 (0.09) | 4.3 (0.1) | **1346 (402)** | 22.2 (4.6) | **48 (25)** | 6.1 (2.2) | **48 (16)** | **27 (3)** | **79 (6)** |
| | Nitrogen × Invasion | 0.76 (0.09) | 3.9 (0.1) | 2307 (558) | 24.8 (7.3) | 153 (34) | 6 (3.3) | **134 (5)** | 43 (13) | 295 (118) |
| | Warming × Nitrogen × Invasion | 0.58 (0.08) | 4.0 (0.2) | **1625 (323)** | 16.8 (2.3) | 89 (27) | 3.1 (0.8) | 34 (9) | 56 (9) | 210 (31) |
| Mineral | Control | 0.26 (0.04) | 4.3 (0.2) | 5859 (2419) | 15.3 (2.9) | 21 (8) | 1 (0.5) | 255 (59) | 15 (2) | 49 (27) |
| | Warming | 0.26 (0.03) | 4.3 (0.0) | **2560 (442)** | 12.4 (3) | 8 (4) | 2.1 (0.8) | 140 (17) | 11 (3) | 32 (13) |
| | Nitrogen | 0.28 (0.03) | 4.2 (0.1) | 4009 (818) | 18.1 (4) | 11 (2) | 1 (0.6) | 661 (383) | 13 (3) | 64 (21) |
| | Invasion | 0.32 (0.05) | 4.2 (0.1) | 5126 (924) | 12.3 (2.1) | 22 (6) | 1.7 (0.6) | 157 (30) | 11 (3) | 38 (11) |
| | Warming × Nitrogen | 0.28 (0.05) | 4.3 (0.1) | 3620 (979) | 15.6 (2.4) | 11 (3) | 1.1 (0.5) | 170 (28) | 10 (2) | 33 (10) |
| | Warming × Invasion | 0.24 (0.03) | 4.4 (0.1) | 3487 (422) | 13.8 (4.8) | 8 (3) | 0.3 (0.2) | 313 (184) | 15 (4) | 112 (54) |
| | Nitrogen × Invasion | 0.37 (0.01) | 4.01 (0.1) | 4124 (538) | 10.4 (1.9) | 13 (4) | 0.7 (0.4) | 87 (2) | 26 (11) | 108 (55) |
| | Warming × Nitrogen × Invasion | 0.3 (0.03) | 4.1 (0.2) | 4077 (1124) | 18.2 (1.6) | 15.8 (3.1) | 0 (0.3) | 169 (6) | 12 (1) | 87 (25) |

*Multiple comparisons were not performed for soil moisture, soil pH, inorganic N contents, or N mineralization because they were not significantly affected by the abiotic treatments, biotic invasion, or their interaction (see Supplementary Table 2.3).

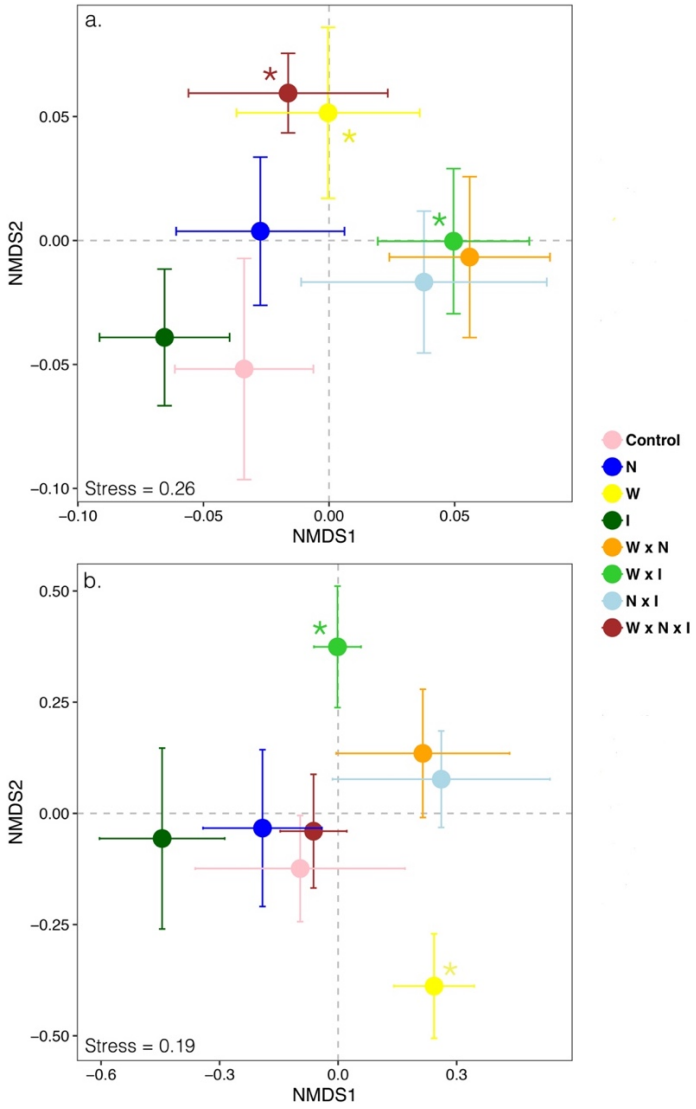


**Figure 2.1.** Fungal community composition across the global change treatments based on fungal ITS2 (a) and AMF 18S (b) metabarcoding. Points represent the average NMDS positions based on Bray-Curtis dissimilarity across both soil horizons, and error bars are the standard error. Asterisks denote significant differences in community composition of treatment relative to control plots (N = nitrogen additions, W = soil warming, I = garlic mustard invasion).

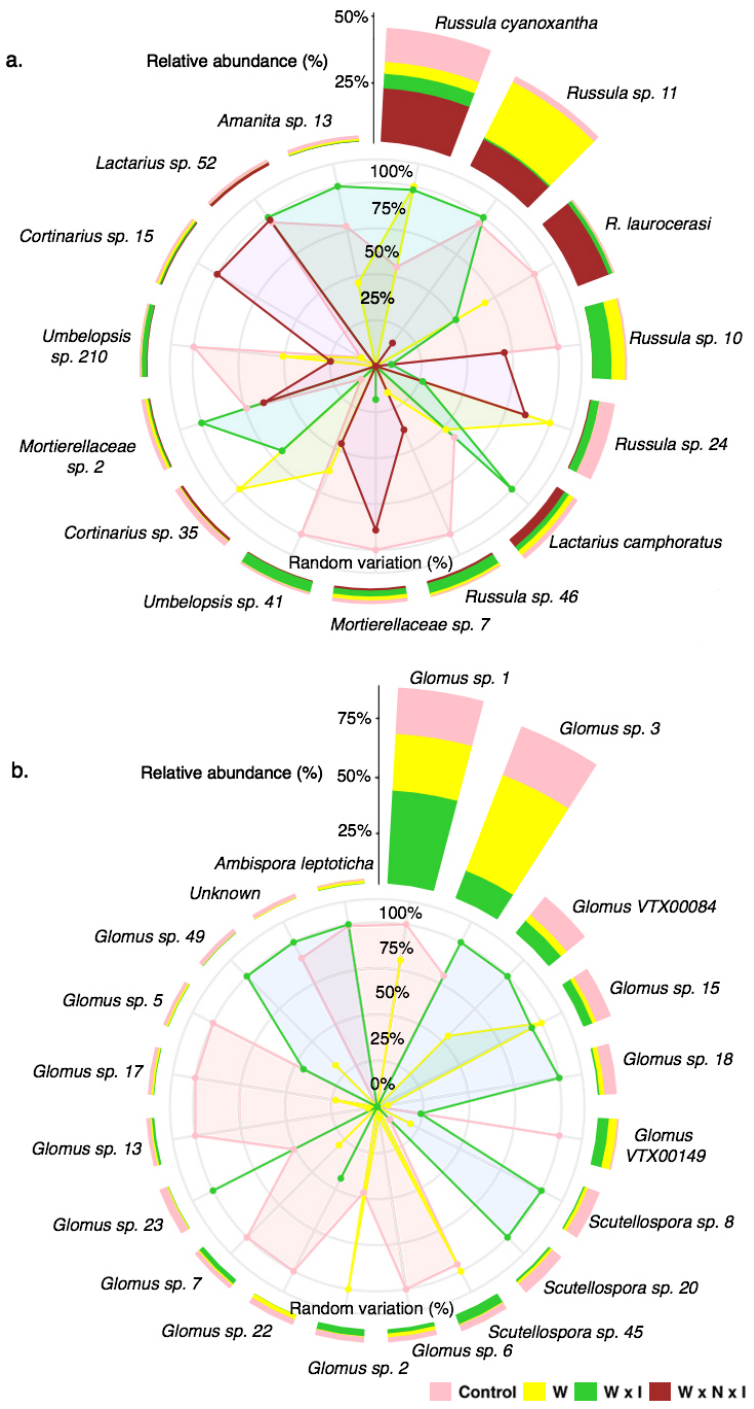


**Figure 2.2**. Relative abundances of the most common fungal (a) and AMF (b) taxa (>1%). Bars represent the mean relative abundance. Points inside represent random variation in fungal relative abundances (*i.e.* variation not accounted for by measured soil parameters). Only treatments where fungal community composition was altered are shown.

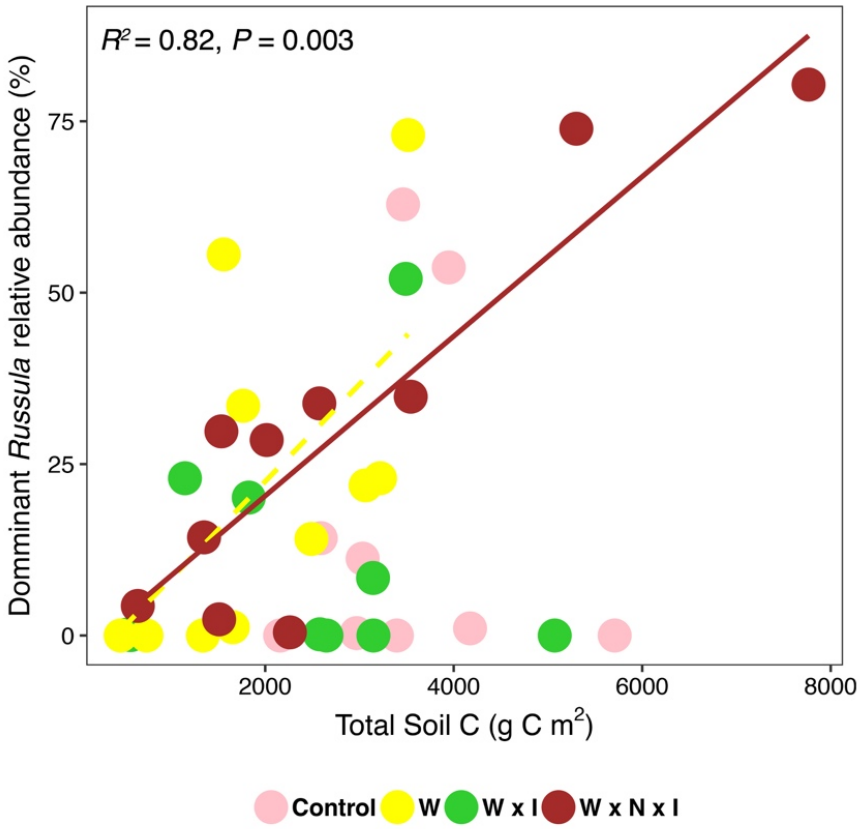


**Figure 2.3.** The correlation between relative abundance of the two dominant *Russula* (*Russula cyanoxantha* + *Russula sp. 11*) and total soil C. This correlation was not significant in the control or W × I plots (no line), approached significance in the W plots ($R^2$ = 0.32, $P$ = 0.08; dashed line), and was significant in the W × N × I plots (solid line). Only treatments where fungal community composition was altered are shown.

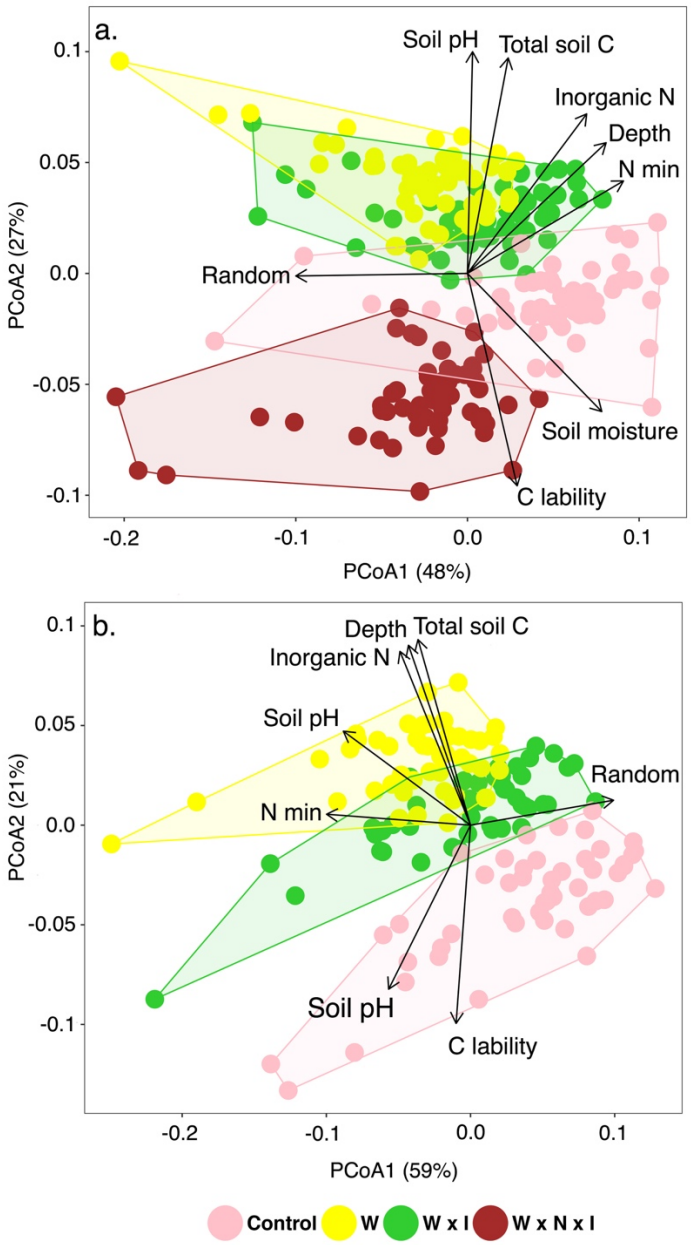


**Figure 2.4.** Fungal (a) and AMF (b) realized niches across the global change treatments. Each point represents the mean Euclidean distance of variation explained by all soil properties, soil processes, and the random variable for a fungal taxon based on joint species distribution modeling and variation partioning. Convex hulls represent the range in species realized niches across treatments, and vectors display correlations for individual soil parameters. Only treatments where fungal community composition was altered are shown.

## Chapter 3

## Indolic glucosinolates maintain the non-mycorrhizal status of *Arabidopsis thaliana*

### 2. Abstract

Being a non-mycorrhizal plant in a mostly mycorrhizal world poses unique challenges. One surprising obstacle is growth suppression by arbuscular mycorrhizal fungi (AMF). The Brassicaceae are a large group of agriculturally important non-mycorrhizal plants that can be colonized by AMF, with negative impacts on plant growth of similar magnitude to that observed for fungal pathogens. Whether Brassicaceae can prevent AMF from colonizing their roots remains unknown. Here we determined, using the model Brassicaceae, *Arabidopsis thaliana,* whether indolic glucosinolates (IGs), produced by all Brassicaceae, protect these non-mycorrhizal plants from AMF colonization. We used well-characterized transgenic lines of *A. thaliana* that have their IG biosynthesis capacity removed entirely or enhanced and challenged them with AMF inoculation. AMF suppressed the performance of IG-removed plants, activated their induced defense system, and colonized their root systems while having no effect on the wild-type or IG-enhanced plants. AMF never highly colonized or activated defenses in wild-type or IG-enhanced plants. Our work suggests that IGs play an essential role in maintaining the non-mycorrhizal status of Brassicaceae and provides novel insight aimed at improving agricultural plant breeding and crop rotation practices to reduce AMF parasitism.

## 3. Introduction

Only 10% of plants are estimated to be non-mycorrhizal (Brundrett & Tedersoo, 2018), but many of these plants are still routinely colonized by arbuscular mycorrhizal fungi (AMF; Hirrel *et al.* 1978, Allen *et al.* 1989, Regvar *et al.* 2003, Rinaudo *et al.* 2010, Veiga *et al.* 2011, 2013). Non-mycorrhizal plants lack numerous symbiosis-related genes that mycorrhizal plants possess (Delaux *et al*. 2013), and AMF colonization suppresses their growth similar to that observed for fungal pathogens (Allen *et al.*1989, Veiga *et al.* 2011, 2013). Some non-mycorrhizal plants have evolved new trophic strategies (*e.g.,* parasites, carnivores) or become habitat specialists, allowing them to generally avoid AMF environments (Brundrett & Tedersoo, 2018). However, some non-mycorrhizal plants still share a similar niche to AMF-associated vegetation and frequently co-occur with AMF (Lambers & Teste, 2013). The Brassicaceae are part of the latter group, and AMF can colonize Brassicaceae roots (Hirrel *et al.* 1978, Regvar *et al.* 2003), reducing plant growth by >50% (Veiga *et al.* 2013). Since the Brassicaceae includes over 4,000 species (The Plant List, 2013), including many agriculturally important crops (*e.g.,* canola white mustard, broccoli, kale, cauliflower), understanding how Brassicaceae may avoid or minimize interactions with AMF could provide novel insight into domestication of plants that deter AMF colonization.

When an arbuscular mycorrhizal plant initiates mycorrhizal symbiosis, conserved signal molecules induce AMF germination and the branching of hyphae towards the root (Bonfate & Genre, 2015). Even non-mycorrhizal Brassicaceae produce some of these signaling molecules (*e.g.* strigalactone) and attract AMF towards their roots, including the non-mycorrhizal plant, *Arabidopsis thaliana* (Brassicaceae; Fernandez *et al.* 2019). *Arabidopsis* lacks subsequent genes involved in mycorrhizal symbiosis that are conserved across plants that form functioning

mycorrhizas (Delaux *et al.* 2013), and an incompatible relationship arises after the first few days of mycorrhizal formation (Fernandez *et al.* 2019). Brassicaceae may fortify their roots against AMF at this stage using phytochemicals known as glucosinolates. There are over 120 glucosinolates (Fahey *et al.* 2001) and while they serve no known primary physiological function, many deter herbivores and have antifungal properties (Zukalová & Vašák, 2002) with important agricultural applications (Fahey *et al.* 2001; Anilakumar *et al.* 2006). Glucosinolates can suppress fungal pathogens (Zhang *et al.* 2015) and the germination of AMF spores (Vierheilig & Ocampo, 1990, Schreiner & Koide, 1993, Cantor *et al.* 2011). Indolic glucosinolates (IGs) are specifically activated alongside various plant defense systems (Clay *et al.* 2009), and unlike other glucosinolates, IGs are not volatile (Zukalová & Vašák, 2002), which may preserve IG concentrations in the root, preventing AMF from developing specialized structures (arbusucles) that mediate a functioning symbiosis. Here, we examined the role of IGs in reducing AMF colonization over the course of the *Arabidopsis* life cycle.

*Arabidopsis* has well annotated IG biosynthesis pathways (Wittstock & Halkier, 2002), and transgenic plant lines have been developed with either a reduced or enhanced capacity to produce IGs (Zhao *et al.* 2002, Celenza *et al.* 2005), making this an ideal model system to test the role of IGs in deterring AMF symbiosis in the Brassicaceae. We inoculated soils of transgenic plants (IG-removed & IG-enhanced) with *Rhizophagus irregularis*, a common, generalist AMF species that has been previously shown to colonize *Arabidopsis* (Veiga *et al.* 2013, Fernandez *et al.* 2019), to determine how IGs mediate associations with AMF. We measured overall plant performance based on growth, height, and reproductive output, as well as AMF colonization and salicylic acid contents. Salicylic acid is a key hormone that guides the expression of induced plant defenses (Dodds & Rathjen, 2010) and if *Arabidopsis* can produce

IGs that deter AMF, then modified plants lacking IG production capacity should have higher salicylic acid activity relative to plants that cannot produce IGs. We had three hypotheses: [1] AMF addition will suppress IG-removed plants more than wild-type plants and will not affect IG-enhanced plants; [2] all plants exposed to AMF will have elevated SA contents, but SA will be highest in IG-removed plants and lowest in IG-enhanced plants; and [3] AMF colonization will be greatest in IG-removed plants and lowest in IG-enhanced plants.

## 3. Materials and Methods

### *3. Plant and fungal strains*

This work was conducted using well-characterized transgenic lines of *Arabidopsis*. IGs are synthesized via tryptophan modification (ATR) and the activity of multiple cytochrome P450 (CYP) dependent mono-oxygenases (Mikkelsen *et al.* 2000; Zhao *et al.* 2002; Celenza *et al.* 2005). CYP of the CYP79 family are encoded by CYP79B2 and CYP79B3 and convert tryptophan to indole-3-acetaldoxime, a precursor IG. Double mutant CYP79B2/CYP79B3 produces undetectable levels of IGs (Zhao *et al.* 2002), hereafter referred to as "IG-removed". ATR genotypes can also influence the expression of CYP79B2 and CYP79B3 (Smolen & Bender, 2002). An overexpression allele, ATR1D, elevates expression of CYP79B2/CYP79B3 (Smolen & Bender, 2002) and enhances IG production (Celenza *et al.* 2005), hereafter "IG-enhanced". The IG-removed (double mutant CYP79B2/CYP79B3) and IG-enhanced plants (ATR1D were derived from wild-type (Col-0) plants (hereafter: wildtype) which produce intermediate IG levels relative to the two mutants (Celenza *et al.* 2005). Detailed information on the plant transformations is available in Zhao *et al.* (2002) and Celenza *et al.* (2005).

Spores of *Rhizophagus irregularis* (fungal culture-line DAOM197198, DAOM181602, and MUCL43194) were obtained from Symplanta (Graupnerweg, Germany). The inoculum contained 1 million spores in 250 grams of diatomaceous earth.

*3. Growth media and plant propagation*

Growing medium was prepared using sterilized (autoclaved 2 times for 36 minutes each at 121°C) Miracle-Gro Premium Potting Mix (Marysville, OH) amended with AMF inoculum in diatomaceous earth or sterilized 100% food grade diatomaceous earth. All treatments had the same ratio of sterilized potting soil and diatomaceous earth (4,000:1). We added AMF at two realistic levels typically observed in field studies: 10 spores per gram of soil (Sieverding *et al.* 1989, Yang *et al.* 2011, Aleixo *et al.* 2014) and 50 spores per gram of soil (Picone *et al.* 2000, Stürmer and Siquiera, 2011).

Plants were grown in individual plastic pots (5 × 5 cm) at 25°C with 50% humidity under 10,000 lumen fluorescent lamps on a 16:8 hour on:off light cycle. Approximately 10 cold stratified seeds were sown on top of six replicate pots per treatment. After one week, we seeded approximately 10 more seeds to each pot to ensure full germination. We allowed multiple seeds to germinate until a single plant produced true leaves, after which point, we continuously weeded the pots to maintain one plant per pot. Plants were bottom fed water 3-4 times a week with 1-10 mL of ultra-purified deionized water.

*3. Measuring performance and harvesting plants*

We tracked the performance of plants over the course of their development. We measured the number of days until germination, flower production, reproductive output, and overall performance. Plant development varied across the treatments, but we sampled plants at the same development stage, which varied between 5-7 weeks. Unless plants died early on or began to die

before producing fruits (siliques), each plant was grown until seeds were visible inside the fruits (siliques) but before the fruits began to senescence. At this point, we measured the height of the plants from the base of the rosette to the highest stem and the number of fruits on each branch as a measure of reproductive output. We then scored the overall performance of plants based on whether they died early (1), began to die before producing siliques (2), began to die after producing siliques (3), were fit (biological and reproductive) with minimal discoloring or wilting (4), or were fit with no discoloring or wilting (5).

We then harvested the plants and split the aboveground and belowground components. A tissue sample from each plant was taken and dried at 60°C for 96 hours and used to calculate moisture content. This dry tissue was then finely ground, and total carbon and nitrogen contents were quantified using dry combustion on a Perkin Elmer 2400 Series II CHN elemental analyzer (Waltham, MA) A separate leaf was taken from each plant, immediately flash frozen in liquid N, and stored at -20ºC for salicylic acid quantification. Primary and lateral roots were collected from each plant, rinsed, and used to measure AMF colonization.

*3. Salicylic acid measurements and AMF colonization*

We measured salicylic acid contents on leaves using a modified spectrophometric procedure (Warrier *et al.* 2013). Frozen leaf tissues were ground in liquid N to a fine powder. Deionized water (1 mL) was added to the ground tissue (50-200 mg) and vortexed for 45 seconds and then centrifuged at 10,000 *g* for 10 minutes. Clear supernatant (9 μL) was added to 1% ferric chloride solution (261 μL) in clear 96-well plates which were then incubated for 5 minutes at 25°C in the dark. Violet color development was analyzed spectrophotometrically at 540 nm and quantified using a salicylic acid standard curve.

We collected roots to quantify AMF colonization using the grid-intersection method (McGonicle *et al.* 1990). Roots were cleared at 90ºC in 10% KOH solution for 20 minutes, acidified in 2% HCl for 15 minutes, and immersed in a 0.03% chlorazol black solution (glycerol, deionized water, and lactic acid; 1:1:1; v:v:v) for 3 hours at 90°C. Stained roots were measured for hyphal, arbuscule, and vesicle colonization after 150 passes at 200× magnification on a compound microscope.

*3. Statistics*

All statistical analyses were conducted in R (R Core Team, 2018) and significance was set at $P \leq 0.05$. We used linear mixed effects models to determine the influence of AMF inoculum density (0, 10, or 50 spores $g^{-1}$ dry soil), plant genotype (IG-removed, wild-type, IG-enhanced), and their interaction on reproductive output (# of fruits per branch), height (cm), aboveground biomass (mg of dry tissue), tissue N content (%), and tissue C content (%). Since there were significant AMF inoculum × genotype effects for the three developmental responses (i.e., reproductive output, height, aboveground biomass; Supplementary Table 3.1), we calculated the difference between sterile (no AMF inoculum) and AMF-amended plants so that the effect of AMF on development could be directly compared across genotypes. Not all plants with AMF inoculation grew large enough to produce sufficient biomass for salicylic acid analysis or AMF colonization, so we combined the 10 and 50 spores $g^{-1}$ dry soil treatments and compared these to the sterile soils as a two-level factor (sterile vs. AMF).

We used the lme function in the nlme package (Pinheiro *et al.* 2007), using replicate as a random variable in all models. Since there were different variances across some levels of the factors (heteroscedasticity), we also created models with unequal variance structures and used these models if they significantly lowered the AIC score relative to the base model. Contrasts

were made using individual *t*-tests with homoscedastic or heteroscedastic variance to test our main hypotheses. Model residuals were inspected for normality using qqnorm plots and Shapiro Wilk tests of normality.

## 3. Results

AMF inoculation reduced the overall performance of IG-removed plants compared to those grown in sterile soils without AMF, while the performance of wild-type and IG-enhanced plants were similar under both sterile and AMF-inoculated conditions (Figure 3.1). AMF inoculation caused half of the IG-removed plants to senesce prior to producing siliques. AMF also reduced the reproductive output on IG-removed plants by ten fruits per branch but had no effect on wild-type plants while increasing reproductive output on IG-enhanced plants by three fruits per branch (Figure 3.2). Plant height response mirrored reproductive output. AMF reduced aboveground biomass of IG-removed plants by 30-175 mg, but this was only significant at the higher inoculation level (50 spores $g^{-1}$ dry soil). AMF did not affect the biomass of wild-type or IG-enhanced plants. There were no differences in tissue C or N contents across genotypes or in AMF inoculated versus sterile soils (Supplementary Table 3.1).

AMF significantly increased salicylic acid content in IG-removed plants but not in wild-type or IG-enhanced plants. Since plant performance of IG-removed plants was so poor with AMF inoculation (Figure 3.1), only a subset of the IG-removed plants produced sufficient tissues for destructive sampling ($n = 5$); therefore, we bulked IG-removed tissues from both AMF inoculation levels (10 and 50 spores $g^{-1}$ dry soil; see *Materials and Methods > Statistics*). IG-removed plants produced more salicylic acid with AMF inoculation compared to sterile soils (Figure 3.2). Conversely, there was no difference in salicylic acid contents between sterile and AMF-inoculated growth environments for wild-type or IG-enhanced plants.

AMF colonization was low (<10%) across all plant genotypes, but it was highest on roots of the IG-removed plants (Figure 3.3). There were few discernable arbuscules and hyphae on any root systems (Supplementary Figure 3.1), but vesicles were observed for all plant genotypes. Vesicle colonization was highest on IG-removed roots with AMF inoculation and was significantly higher than wild-type or IG-enhanced plants (Figure 3.2). Wild-type and IG-enhanced plants were never highly colonized by AMF regardless of growth environment (sterile vs. AMF inoculation).

SA content was correlated to plant biomass for wild-type and IG-enhanced plants but not IG-removed plants (Table 3.1; Supplementary Figure 3.2). Wild-type and IG-enhanced plant biomass were positively correlated to SA contents when grown with AMF, but not in the sterile soil treatment. Neither reproductive output nor plant height (Figure 3.3) were correlated to SA content or vesicle colonization for wild-type or IG-enhanced plants. Plant height was positively correlated to SA contents for IG-removed plants but not reproductive output. Vesicle colonization was not correlated to plant height or reproductive output for any plants.

## 3. Discussion

We found that the ability to produce IGs deterred AMF colonization on the roots of the non-mycorrhizal *Arabidopsis*, suggesting that IGs serve an important and previously unknown ecological function whereby they counter AMF-induced suppression of non-mycorrhizal plant growth. AMF sharply reduced the performance of plants that could not produce IGs. We hypothesize that this extends beyond *Arabidopsis* since AMF can colonize many other species in the Brassicaceae (Hirrel *et al.* 1978), and other AMF taxa besides *Rhizophagus irregularis* can colonize non-host plants (Regvar *et al.* 2003). If Brassicaceae are grown in soils after or alongside AMF host plants then the IG production capacity of the plant should be considered. A

major goal of Brassicaceae breeding has been to reduce glucosinolate levels because they can decrease palatability and cause thyroid and liver damage, the latter being of particular concern for crops (e.g., canola) whose byproducts are used in animal feed (Fahey *et al.* 2001; Anilakumar *et al.* 2006). There may be a tradeoff between yields and the palatability/toxicity of agricultural Brassicaceae that should be compared for optimum plant breeding. Compared to AMF associated cereals and legumes, the root systems of Brassicaceae tend to be deeper and different pathogens infect Brassicaceae, so they are frequently used in intercropping and to diversify crop rotations alongside mycorrhizal plants (Singh *et al.* 2010), but in soils where AMF spores are commonly found (10-50 spore $g^{-1}$ dry soil), low IG Brassicaceae (*e.g.,* canola; Shahidi *et al*. 1989) may be suppressed by AMF.

We found partial support for our first hypothesis that AMF would suppress the performance of IG-removed plants with reduced impacts on wild-type and IG-enhanced plants. AMF inoculation reduced the performance of IG-removed plants to the point of preventing reproduction before the onset of senescence. However, there were no effects of AMF amendment on wild-type plants. Since minute concentrations of glucosinolates can suppress AMF germination (Cantor *et al.* 2011), the impacts of AMF on *Arabidopsis* may not scale with IG production within the range we detected. The IG-removed plants produce undetectable levels of IGs (Zhao *et al*. 2002), while the IG-enhanced plants produce 14× more IG than wild-type plants (Celenza et al. 2005). Some commonly grown canola cultivars already produce 10× the IG levels of low IG cultivars (Shahidi *et al*. 1989), so it may be advantageous to use higher IG cultivars in soils where AMF plants have been previously or are currently being grown. Surprisingly, the IG-enhanced plants had higher reproductive output and grew taller with AMF inoculation. Although it would be striking, we doubt the positive AMF effect in IG-enhanced plants was because of a

functioning arbuscular mycorrhiza, especially because tissue N contents did not increase with AMF additions.

Our second hypothesis was that AMF colonization would stimulate salicylic acid production in plant tissues of all three genotypes (IG-removed plants < wild-type < IG-enhanced plants. Salicylic acid mediates key regulatory roles in induced plant defenses (Dodds & Rathjen, 2010) and accumulates in pathogen infected tissues (de Vos *et al.* 2005). We observed increased salicylic acid content in IG-removed plants exposed to AMF, which supports the prediction that AMF presence would activate plant defense systems in IG-removed plants. These plants may have over-invested in induced defenses, and this could be why their growth was suppressed by AMF inoculation (Allen *et al.* 1989). Genotypes that produce IGs (*i.e.,* wild-type and IG-enhanced) did not exhibit significantly elevated tissue salicylic acid contents. We hypothesize that this was a direct result of lower AMF root colonization on these genotypes. There was a positive correlation between salicylic acid production and wild-type and IG-enhanced plant biomass in soils with AMF inoculation, but this was not observed for IG-removed plants. Plants with IG-producing capacity likely invested the appropriate amount in induced defenses, minimizing AMF colonization and increasing plant biomass.

Finally, we hypothesized that AMF colonization would be highest on IG-removed plant roots, followed by wild-type and IG-enhanced plants. This hypothesis is supported by the observation that IG-removed plants were most heavily colonized by AMF while IG-producing plants (i.e., wild-type, IG-enhanced) were not highly colonized. This observation suggests that that IGs deter AMF colonization on Brassicaceae roots. Consistent with other work, root arbuscules were rare on all plant genotypes, with vesicles being the most common root structure observed (Schreiner & Koide, 1993, Regvar *et al.* 2003, Veiga *et al.* 2013). The vesicles we

observed (Supplementary Figure 3.1) are morphologically similar to vesicles found on other Brassicaceae (Regvar *et al.* 2003) and are presumably not a sign of a functioning mycorrhiza.

Our results provide evidence that IGs facilitate resistance to AMF colonization in *Arabidopsis,* which we presume likely holds for other Brassicaceae. IG production is conserved amongst Brassicaceae, and most other plant families that produce IGs are members of the Brassicales (order), so there is a phylogenetic signal to their biosynthesis (Fahey *et al.* 2001). Other members of the Brassicales that can form arbuscular mycorrhizas (i.e. Tropaeolaceae) also produce significantly less IGs compared to Brassicaceae (Vierheilig *et al.* 2000), lending further support to this hypothesis. Future would should consider how the relationship between glucosinolates and myrosinase affects interactions with AMF since myrosinase may co-evolve with glucosinolates as it degrades glucosinolates into isothiocyanates, which are also fungicidal (Fahey *et al.* 2001). We hypothesize that the evolution of indolic glucosinolates is one mechanism by which plants maintain a non-mycorrhizal lifestyle.

**Table 3.1.** Linear regressions between plant performance and salicylic acid contents and AMF vesicle colonization in the AMF inoculation treatments. Significant values are in bold and positive and negative correlations are indicated using '+' and '–' symbols, respectively. Correlations are displayed in Supplementary Figure 3.2.

| Predictor variable | Reproductive output (# of fruits per branch) | Height (cm) | Biomass (mg dry tissue) |
|---|---|---|---|
| **Salicylic acid** ($\mu g\ g^{-1}$ dry tissue) | | | |
| IG-removed | $R^2 = 0.51$, $P = 0.29$ | ***R2 = 0.84, P = 0.008* (+)** | $R^2 = 0.04$, $P = 0.8$ |
| Wild-type | $R^2 = 0.06$, $P = 0.5$ | $R2 = 0.18$, $P = 0.2$ | ***R²* = 0.39, *P* = 0.04 (+)** |
| IG-enhanced | $R^2 = 0.21$, $P = 0.18$ | $R2 = 0.19$, $P = 0.2$ | ***R²* = 0.51, *P* = 0.02 (+)** |
| **AMF vesicle colonization** (%) | | | |
| IG-removed | $R^2 = 0.05$, $P = 0.77$ | $R^2 = 0.11$, $P = 0.58$ | $R^2 = 0.31$, $P = 0.33$ |
| Wild-type | $R^2 = 0.07$, $P = 0.42$ | $R^2 = 0.13$, $P = 0.27$ | $R^2 < 0.01$, $P = 0.86$ |
| IG-enhanced | $R^2 = 0.23$, $P = 0.17$ | $R^2 < 0.01$, $P = 0.85$ | $R^2 = 0.29$, $P = 0.11$ |

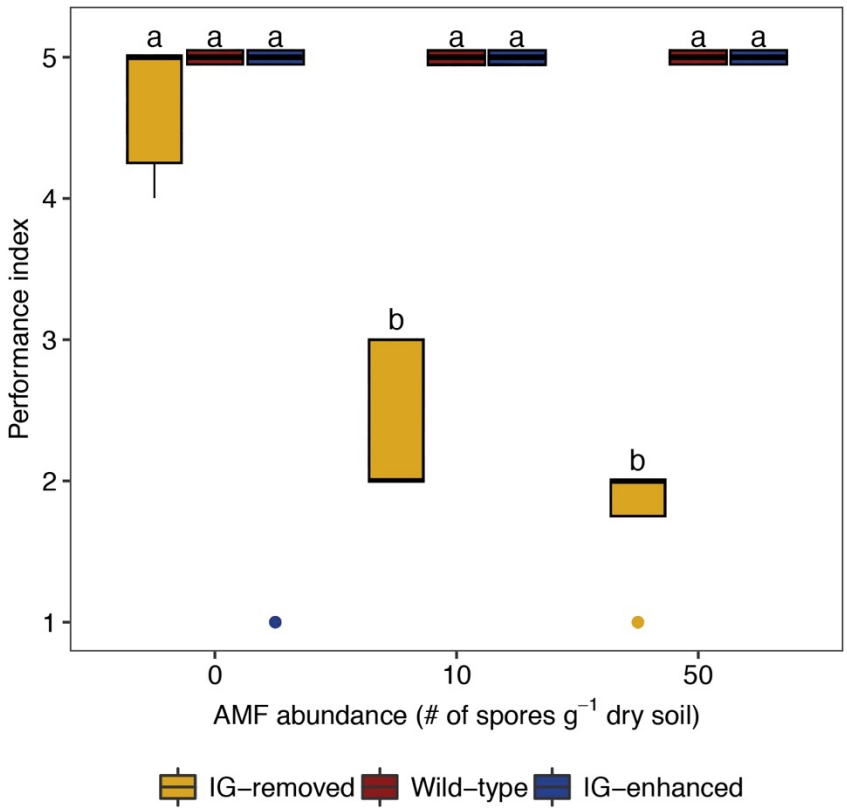


**Figure 3.1.** Performance index across plant genotypes in response to varying AMF inoculation levels. Performance values represent plants that died before bolting (1), began dying before reproduction (2), began dying after reproduction (3), were fit with little discoloring or wilting (4), and were very fit with no discoloring or wilting (5). Bars represent the interquartile range, whiskers show the minimum and maximum values, points show suspected outliers, and the horizontal black lines show the median. The wild type and enhanced plants had the same value (5) across all replicates minus one suspected outlier. Bars with different letters are significantly different ($P \leq 0.05$).

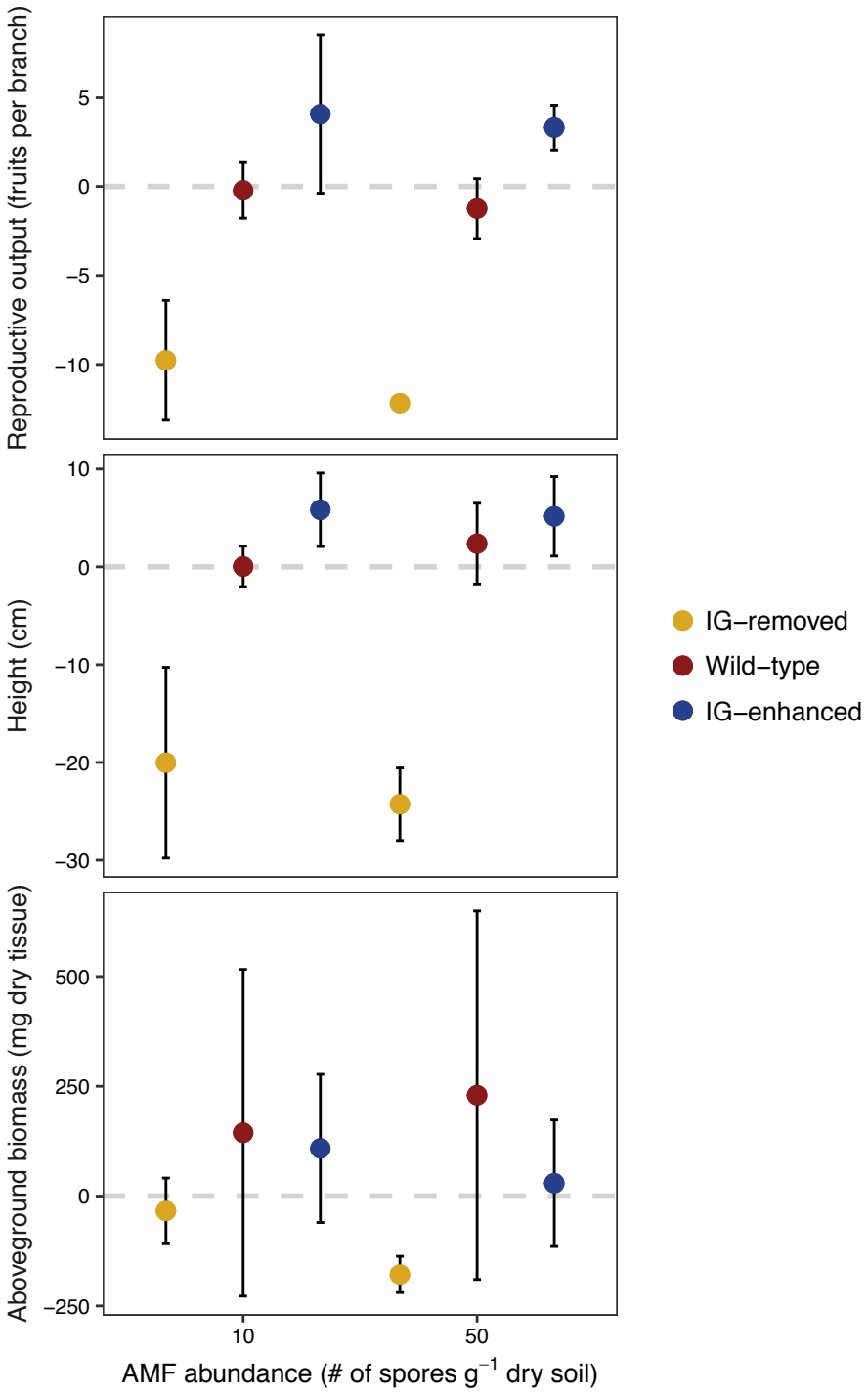


**Figure 3.2.** Plant genotype performances across AMF inoculation levels. Values represent difference between inoculated and sterile controls ($\text{treatment}_{Genotype\ replicate} - \text{sterile}_{Gentoype\ mean}$). Points represent the mean difference and error bars are the standard deviation. Standard deviations that do not pass through zero are significantly different.

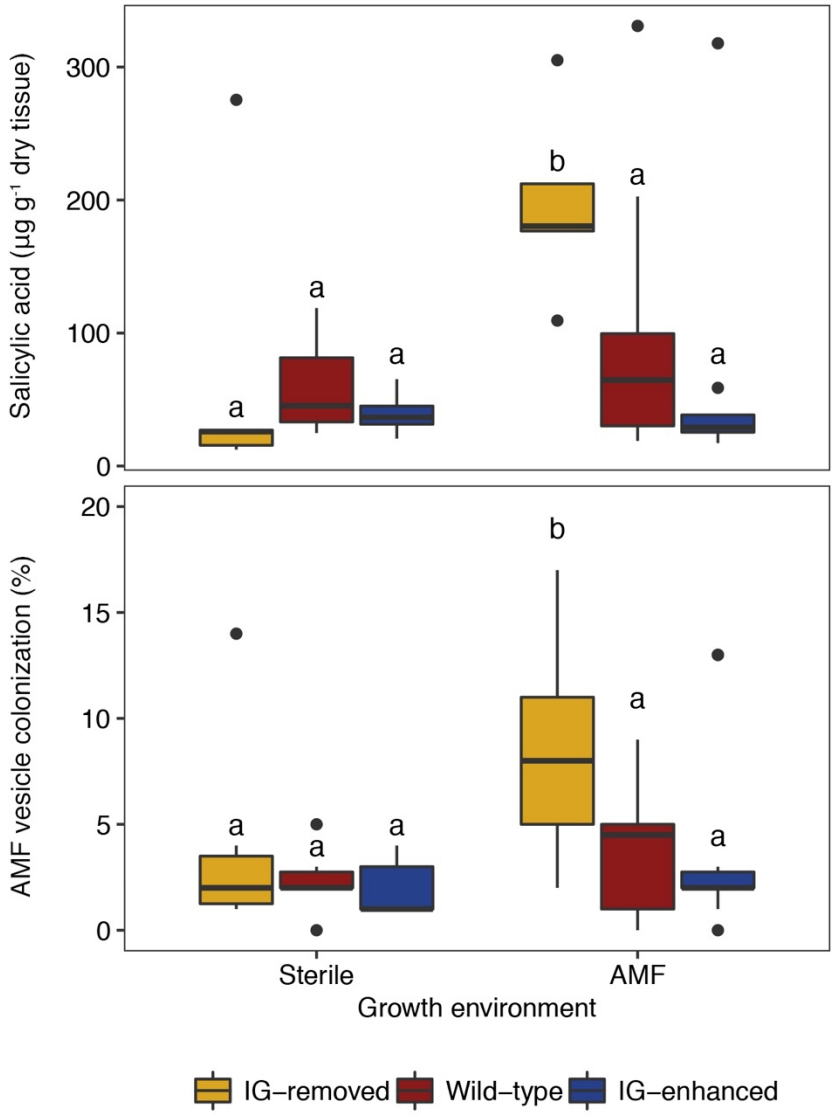


**Figure 3.3.** Salicylic acid content and AMF vesicle colonization in sterilized soil (no AMF) versus soil with AMF inoculation. Bars represent the interquartile range, whiskers show the minimum and maximum values, points show suspected outliers, and horizontal black lines show the median. Bars with different letters are significantly different ($P \leq 0.05$).

# Chapter 4

# Chronic dispersal and recruitment limitations are the primary driver of soil fungal community assembly

## 3. Abstract

Fungal communities frequently vary in consistent ways across environmental (*i.e.,* edaphic, floral) gradients. However, there is often more unaccounted for variation in communities than can be explained by environmental conditions, indicating that neutral processes may sometimes be more important than niche processes in structuring fungal communities. Site conditions (*e.g.,* geography, climate, edaphics) and the relative dominance of fungal functional guilds could explain the balance between niche and neutral processes, but this has never been compared across large spatial scales. In this study, we re-analyzed two global surveys of fungal communities (Tedersoo *et al*. 2014; Davison *et al*. 2015) and asked whether certain environmental properties or fungal guilds were associated with particular proportions of niche versus neutral assembly we used compositional null models to evaluate community assembly. we found that in 91% of the plots across the globe fungal assembly was driven primarily by stochastic processes indicative of chronic dispersal and recruitment limitations. Yeasts and plant pathogens had the most idiosyncratic assembly. At <10% of sampling locations, fungal assembly was primarily related to niche processes and correlated with environmental properties. Niche assembly was observed in tundra, boreal, and northern temperate systems but only for ectomycorrhizal, arbuscular mycorrhizal, and general saprotrophic fungi. we therefore show using two independent datasets that fungal assembly is primarily stochastic at sites across

the globe, but in northern latitudes, a consistent group of taxa organizes around environmental conditions via niche processes.

## 4. Introduction

Fungi can seek out and exploit exceedingly limiting nutrients, a trait that allows them to inhabit virtually every habitat across the globe (Webster and Weber, 2007) but with considerable variation in community composition for reasons that are only beginning to be understand (Tedersoo *et al*. 2014, Davison *et al*. 2015). Dispersal limitations can directly affect fungal community composition (Lekberg *et al*. 2007, Peay *et al.* 2012, Bahram *et al.* 2016, Glassman *et al.* 2017a), and alongside other neutral processes (*i.e.,* recruitment limitations, drift), dispersal limitations can be a powerful force that shapes community composition (Bahram *et al.* 2016, Powell *et al.* 2015, Powell and Bennet, 2016, Zhou and Ning, 2017). At the same time, site temperature and precipitation (Tedersoo *et al.* 2014, Miyamoto *et al.* 2017), land-use (Andrew *et al.* 2019, Davison *et al.* 2015), elevation (Nottingham *et al.* 2018), plant diversity (Tedersoo *et al.* 2014, Andrew *et al.* 2019), edaphic conditions (Kivlin *et al.* 2014, Glassman *et al.* 2017b, Thomson *et al.* 2015), disturbance (Caruso *et al.* 2012), and global change exposure (Lilleskov *et al.* 2011, Andrew *et al.* 2018) also shape the fungal community. The balance of environmental selection based on species niches relative to neutral processes like random dispersal, drift, and speciation is poorly understood for fungi and often not measured (Chaudbury *et al*. 2008, Zhou and Ning, 2017). Yet with a back drop of unprecedented global climate change (Halim *et al.* 2018), the response and conservation of fungi to future conditions requires an understanding of the current assembly processes that structure fungal communities.

Niche processes tend to produce predictable community compositions while neutral processes produce stochastic compositions (Mori *et al*. 2013, Powell *et al*. 2015), and there is

evidence that arbuscular mycorrhizal fungi (AMF) primarily assemble via stochastic processes (Powell and Bennet, 2016). At mid- and southern latitudes, AMF assembly is primarily stochastic but becomes more predictable and niche-based at higher latitudes for reasons that are not yet clear (Powell and Bennet, 2016). AMF communities also assemble less stochastically in undisturbed sites compared to disturbed land use types (Caruso *et al*. 2012), which suggests that there are important geographic (*e.g.* latitude) and environmental (*e.g.* land-use) parameters that can shape fungal assembly. Less is known about the assembly of other fungal lineages and functional groups, though saprotrophs and ectomycorrhizal fungi (EMF) exhibit different small-scale distance-decay patterns (Bahram *et al*. 2016) and large-scale co-variation with environmental parameters (Tedersoo *et al.* 2014). At larger spatial scales, fungal assembly will presumably vary across environmental conditions since the relative abundance and richness of functional guilds changes across biomes and environmental gradients (Tedersoo *et al*. 2014), but no study has examined the underlying assembly processes of fungal guilds across large spatial scales.

Here we used null modeling to heuristically disentangle the drivers of fungal community assembly at plots located across the globe (Azeria *et al*. 2009). Briefly, compositional null models ask a simple question: from a species pool, are species that form communities associated with particular environments more similar to each other than a random draw of species from the species pool (Azeria *et al*. 2009). If site conditions select for particular fungal taxa then we would expect communities to be more similar than a random selection of species from the species pool (Caruso *et al*. 2012) consistent with the Baas-Becking hypothesis that environmental selection is important while dispersal limitations are not for microbes (Baas-Becking, 1934). Random dispersal and recruitment limitations and drift can mask the influence

of site conditions, causing species to form communities indistinguishable from a random selection of species indicative of neutral assembly (Zhou and Ning, 2017). Communities can also be more dissimilar than a random selection of species because of chronic (versus random) dispersal limitations (Powell et al. 2015), biotic interactions (Chase, 2010), and if environmental heterogeneity is higher than expected (Caruso *et al.* 2012). Using null models, we measured assembly processes of belowground (*i.e.,* soil and root) fungal communities, and we determined whether there were geographic, climatic, edaphic, or floral conditions that gave rise to specific assembly processes. This work was conducted on two publicly available datasets collected from a global set of plots to characterize fungal and AMF community assembly (Tedersoo *et al*. 2014, Davison *et al*. 2015; also see re-analyses by Davison *et al*. 2016 and Powell and Bennet, 2016). we calculated an effect size as the difference between the actual community composition and that generated by the null models, and regressed effect size against a large suite of environmental predictor variables. An effect size of -1 indicates complete importance of environmental selection and niche assembly consistent with the Baas-Beckings hypothesis, an effect of 0 fits a neutral model, while an effect size of 1 indicates complete importance of chronic dispersal and recruitment limitations, with values in between -1 and 1 a proportion of these different processes.

## 4. Methods

### *4. Dataset descriptions*

This analysis was conducted on existing datasets made publicly available by Tedersoo *et al*. (2014) and Davison *et al*. (2015). Tedersoo *et al*. (2014) characterized total fungal communities at a global set of sites, while Davison *et al*. (2015) focused exclusively on AMF. Tedersoo *et al*. (2014) sampled 365 sites across the globe, collected soils to a 5 cm depth, and used established DNA metabarcoding procedures to characterize soil fungal communities. The proportion of

fungal operational taxonomic units (OTUs) based on 98% sequence similarity of ITS rDNA was quantified. Accompanying metadata for each plot includes biome, latitude, longitude, altitude, forest age (if at a forested site), basal tree area (if trees present), site area, number of ectomycorrhizal tree species if present, net primary productivity, potential evapotranspiration, mean annual precipitation (MAP), mean annual temperature (MAT), soil pH, total carbon (C), nitrogen (N), and phosphorous (P) content, soil $\delta^{13}C$ and $\delta^{15}N$. and soil potassium, magnesium, and calcium contents. We analyzed the entire dataset and then split it into functional guilds, separating out total saprotrophs and EMF. There were more zeros and lower relative abundances for yeasts, white rot fungi, and plant pathogens compared to general saprotrophs and EMF, so we did not create separate models for these groups because we could not simulate sufficient variation in the null models of community composition relative to actual community composition. We included AMF in this component of the study by incorporating data from Davison *et al*. (2015). Davison *et al*. (2015) surveyed AMF communities at 66 sites spanning the globe and on the roots of 912 different AMF host plants. This study included coarse resolution metadata (site level versus plot) and was also sparser than Tedersoo et al. (2014), so analysis of the Davison et al. 2015 dataset was used to compare across categorical trophic guilds, ecosystem types, and host plants but was not the focus of this study. To examine the potential influence of plant traits on AMF communities, we also linked three commonly measured host plant traits (leaf area, maximum height, minimum height) to AMF assembly. We assigned traits to host plants using the TR8 database and the R package TR8 (Bocci *et al*. 2015).

*4. Null model construction*

Null models were created in R 3.0.2 (R Development Core Team, 2008). First, we randomly rarified the dataset to the lowest sequencing depth 1,000 times using the rrarefy function in

vegan (Oksanan *et al*. 2013). We then calculated Bray-Curtis dissimilarity based on relative abundance of OTUs using the *vegdist* function. Next, 1,000 null models were generated using the rarified OTU table and the *permatfull* function. The OTU matrix was randomized using Patefield's (1981) algorithm. We constrained the null models by holding column (species) sums constant to make sure that abundant and rare species did not switch roles in ecologically unrealistic ways (Mori *et al*. 2015). After creating the null models, we calculated mean Bray-Curtis dissimilarity for each null simulation matrix ($Bray_{null}$) and the standard deviation around these means ($Bray_{sd}$). We then subtracted observed Bray-Curtis dissimilarity from $Bray_{null}$ to calculate beta diversity independent and beyond chance ($Bray_{ind}$). We then calculated an effect size dividing $Bray_{ind}$ by $Bray_{sd}$ and bounded this between -1 and 1 using the minimum and maximum $Bray_{ind}$.

We also created two types of models in order to tease apart whether species membership (*a.k.a.* presence or absence) or relative abundances more strongly influenced assembly patterns. Communities can diverge because they share few species (Sørensen, 1948) or because species relative abundances vary across sites (Bray and Curtis, 1957). Different environmental conditions can influence both membership and abundance (Mori *et al*. 2013), but if few species are shared this is frequently the result of dispersal limitation (Peay *et al*. 2012) and/or competitive exclusion (Diamond, 1975), while relative abundances vary because of recruitment limitations and mortality, with biotic interactions nested in these processes (Hughes, 1986). We therefore created the same null models using Sørensen dissimilarity, which is calculated using the same math as Bray-Curtis, but as the proportion of shared species between sites based on presence or absence (Sørensen, 1948). We ran statistics on the Bray-Curtis model results because this better reflects empirical community composition (Mori *et al*. 2015).

*4. Statistics*

All statistical analyses were conducted in R with significance set at $P \leq 0.05$. we calculated environmental dissimilarity as the Euclidean distance of all environmental variables using the vegdist function in vegan. For environmental dissimilarity and independent environmental variables, we used regression to determine the environmental predictors of effect size. We fit linear and non-linear line types to the data based on model fit (Supplementary Table 4.1). We only report non-linear model statistics for those that had the highest linear correlations but were not linear fits based on scatterplots. To test the influence of discrete variables (biome type, fungal guild) on effect size, we used non-parametric Kruskal-Wallis rank sum tests. For fungal guilds, we compared saprotrophs as a whole and EMF from the Tedersoo *et al*. (2014) dataset but we did not compare this to AMF from Davison *et al*. (2015).

## 4. Results

Effect size, which represents a continuum of assembly processes with purely stochastic assembly at 1, neutral assembly at 0, and environmental selection and niche assembly at -1 (Caruso et al. 2012, Powell and Bennet, 2016), was positive in 91% of the plots across the globe. The median effect size was 0.08, and the maximum and minimum effect sizes were 0.1 and -0.72, respectively. Effect size also varied across biomes ($\chi^2 = 198$, df = 10, $P < 0.0001$; Figure 4.1). Effect size was negative in 9% of the plots, and these plots were primarily in arctic tundra and boreal forests (Figure 4.1). Effect size also varied across fungal guilds and was generally lower for EMF compared to saprotrophs ($\chi^2 = 1{,}575$, df = 1, $P < 0.0001$). EMF and total saprotrophs had negative median effect sizes in arctic tundra and boreal forests (Figure 4.1). For AMF, which we studied separately using the Davison *et al*. (2015) dataset, in 95% of plots, effect size was positive, while in 4.5% of the plots it was negative. Notably, there were a large

number of zeros in this dataset compared to the Tedersoo et al. (2014) dataset, and this is likely why effect size was less variable for AMF compared to EMF and total saprotrophs. Negative effect sizes for AMF were found primarily in forested ecosystems with low MAT (5-10ºC; Supplementary Figure 4.1). Of the AMF host plant traits we tested, minimum plant height was positively correlated to effect size ($R^2 = 0.25$, $P = 0.03$, Supplementary Figure 4.2).

Total fungal community effect sizes further varied along continuous environmental gradients (Supplementary Table 4.1). Effect sizes were negative in plots at high latitudes (35-70ºN; $R^2 = 0.57$, $P < 0.0001$), freezing and cold MAT ($R^2 = 0.42$, $P < 0.0001$), high soil C:N ratios (C:N > 28; $R^2 = 0.2$, $P < 0.0001$), and low soil pH (<3.5; $R^2 = 0.1$, $P < 0.001$; Supplementary Figure 4.3), which were characteristic of arctic tundra, boreal forests, and some of the northern temperate coniferous and deciduous forested sites. Effect size was also positively correlated to overall environmental dissimilarity (Figure 4.2A; $R^2 = 0.31$, $P < 0.0001$); however, this correlation was driven by negative effect sizes. Negative effect sizes were positively correlated to environmental dissimilarity when examined independently (Figure 4.2A; $R^2 = 0.45$, $P < 0.001$), while positive effect sizes were not correlated to environmental dissimilarity. Environmental dissimilarity was also lowest across plots in the arctic tundra and boreal forests and highest across plots in tropical ecosystems (Figure 4.2B; $\chi^2 = 208$, df = 10, $P < 0.0001$). In boreal forests, there was variation in effect size along a net primary productivity (NPP) gradient, with the lowest effect sizes from plots with low NPP (Figure 4.3). NPP was also more strongly correlated to saprotrophic effect size (Figure 4.3; $R^2 = 0.77$ $P < 0.0001$) than EMF effect size ($R^2 = 0.33$, $P < 0.003$), and it was more negative for saprotrophic fungi in plots with low NPP than for EMF. NPP was also positively correlated to effect size in plots in Mediterranean ecosystems but not in other biomes (Supplementary Figure 4.4).

Individual biome analyses showed a larger proportion of negative effect sizes (27% of the plots) compared to the global model (9%), indicating less stochasticity at smaller spatial scales when the species pool is reduced to only taxa found within a biome. In boreal ($R^2$ = 0.6, $P$ < 0.001), temperate coniferous ($R^2$ = 0.22, $P$ = 0.003), and temperate forests ($R^2$ = 0.3 $P$ < 0.001) forests, effect sizes were negative at higher latitudes, mirroring results at the global scale (Supplementary Table 4.2). In dry tropical forests, moist tropical forests, and montane tropical forests, effect sizes did not vary across latitude, but did vary in relation to longitude, though not very strongly ($R^2$ = 0.21-0.28; Supplementary Table 4.2). In Mediterranean ecosystems both latitude and longitude were correlated to effect size, but in Mediterranean ecosystems, low NPP and low soil P content were associated with low effect sizes ($R^2$ = 0.5, $P$ < 0.0001). In Savannas, longitude was correlated to effect size with high soil C:N ratio also associated with low effect sizes ($R^2$ = 0.39, $P$ = 0.0002), but this was multicollinear with altitude ($R^2$ = 0.48, $P$ < 0.0001) and impossible to tease apart. In general, there was not strong co-variation between effect size and environmental variables across most biomes.

Finally, we found that effect size was mostly positive in plots based on null models of presence or absence (Sørensen) in addition to relative abundance (Bray-Curtis; 68%; Figure 4.4). Effect sizes were more positive based on abundance than membership (Figure 4.4). A smaller fraction of effect sizes (23%) were negative based on membership and positive based on abundance (23%). There were no communities with negative effect sizes based on abundance and positive based on membership. The same 9% of communities with negative effect sizes based on abundance were negative based on membership, though effect size was more negative based on membership than abundance.

## 4. Discussion

Fungal community assembly was primarily stochastic in 91% of sampling locations globally and in 73% of plots sampled within specific biomes. Stochasticity, indicated by positive effect sizes, could be related to extensive niche partitioning at environmentally heterogeneous sites (Caruso *et al*. 2012); however, there was no evidence for a niche divergence explanation. Positive effect sizes were consistent across different environmental conditions and did not increase with environmental heterogeneity (Figure 4.2B). Positive effect sizes are therefore unlikely the result of extensive niche partitioning and environmental heterogeneity, and instead, are more likely the outcome of chronic dispersal and recruitment limitations (Powell *et al*. 2015, Powell and Bennet, 2016). The biome-level analyses also support this conclusion since environmental variables were not strongly correlated to effect size within most biomes, though there were more sites with negative effect sizes at the biome scale compared to the global scale, indicating greater detection of niche-based assembly within smaller spatial scales. Since fungal community composition has previously been shown to have high residual variation that cannot be accounted for by environmental characteristics (Bruns *et al*. 2019), these results provide the first clear evidence indicating that this is the result of generally stochastic fungal assembly.

Stochastic assembly can arise because of idiosyncratic species presence or absence (membership), relative abundances, or both (Mori *et al*. 2015). Species membership reflects dispersal and extinction processes with species present if they dispersed into the site (or if speciation occurred) and absent if they did not disperse or went extinct. In contrast, abundance is continuous and reflects recruitment and mortality (Hughes, 1986). In this study, we used a simple, novel framework that compares assembly based on membership to relative abundance in order to disentangle some of these processes (Figure 4.4). Community assembly was more

stochastic when we accounted for relative abundance compared to membership alone, and for 23% of the plots effect size was only stochastic for relative abundance, indicating predictable dispersal and extinction but unpredictable recruitment and mortality. We hypothesize that stochastic relative abundances are central to the co-existence of fungal taxa since there is extensive functional overlap across many fungi (Louca *et al*. 2018) that could result in competition to exclusion (Diamond, 1975). For example, chronic recruitment limitations can reduce relative abundance and alleviate competition, allowing inferior competitors to remain part of a community and maintain co-existence (Hurtt and Pacala, 1995). So far, there have been no temporal analyses of fungal communities at sites across the globe, but this would help to directly disentangle recruitment processes.

A smaller proportion of the communities had negative effect sizes indicative of mostly niche-based assembly. The highest degree of niche-based assembly occurred in northern latitude, cold ecosystems in tundra, boreal forests, and northern temperate forests (Figure 4.1). AMF communities also exhibited niche-based assembly in forests with freezing and cold MAT (Supplementary Figure 4.1). Individual biome models also indicate that fungal communities were assembled more via niche processes in boreal, temperate coniferous, and temperate deciduous forests at northern latitudes. We therefore show, using two independent fungal datasets, that fungal assembly is less stochastic at northern latitudes and more related to niche processes than in other parts of the world. A reasonable explanation for this finding is that there are fewer taxa in northern latitudes, so all communities look similar, but there was no relationship between total fungal richness and effect size (Supplementary Figure 4.5). Rather, negative effect sizes were correlated to environmental dissimilarity (Figure 4.2A). Environmental dissimilarity was also lowest in tundra and boreal forests (Figure 4.2B). In these

biomes, we can therefore deduce that communities converged around similar environmental conditions, indicative of restricted niche-based assembly (Powell *et al*. 2015). For AMF, negative effect sizes were associated with low AMF species richness (<20 taxa), though this was not linear since a greater number of communities with equally low richness levels also had positive effect sizes (Supplementary Figure 4.5). Of the AMF host plant traits we tested (maximum plant height, minimum plant height, leaf area) minimum plant height was positively correlated to AMF effect size, with the most stochastic assembly on plant roots with the highest minimum heights (Supplementary Figure 4.2). Plant height tends to decrease from the equator to the poles (Moles *et al*. 2009). We therefore hypothesize that AMF communities assemble more via niche processes at northern latitudes because plant heights tend to be smaller and because fungal richness is lower.

Community assembly can influence biogeochemical cycles since it directly affects the number of functionally adapted taxa in a community (Graham and Stegen, 2017). Negative effect sizes can indicate that taxa are adapted to environmental conditions since it is a measure showing that these fungal communities are consistency found within these types of environments (Azeria *et al*. 2009). Adaptation could be related to the peak in fungal functional diversity previously observed in northern latitude boreal and tundra biomes compared to other parts of the world (Bahram *et al*. 2018). In these ecosystems, the relative abundance of EMF is also high and comparable to that of saprotrophic fungi (Tedersoo *et al*. 2014), and both fungal groups exhibited overall negative median effect sizes in these biomes. In boreal forests, EMF and saprotrophs had similar effect sizes (Figure 4.1), but low NPP promoted lower effect sizes for saprotrophic fungi compared to EMF. This could indicate a switch in the most adapted functional group (Azeria *et al*. 2009) since low NPP coincides with low belowground C allocation (Gower *et al*. 2001), and

this could negatively affect EMF (Fernandez *et al*. 2017). This could have important implications for soil C cycling via saprotrophic metabolism (Averill *et al*. 2016).

In conclusion, these results show that soil fungi have highly stochastic community assembly patterns primarily as a result of stochastic differences in taxonomic relative abundances. We hypothesize that this is largely due to chronic recruitment limitations. Fungal demography has been likened to vascular plants (Bruns, 2019), which suggests that fungi must increase abundances dramatically to remain stable community members, and this rarely occurs in nature (Tilman, 2004). Fungi frequently have highly uneven community structure with a large tail of rare species that reflects a widespread inability to increase recruitment (Dumbrell *et al.* 2010). Understanding how recruitment limitations influence the co-existence of fungi is an important next step. How assembly influences fungal function in soils is also an important and largely unexplored next frontier. We interpret these results to indicate that fungal communities in northern latitude ecosystems may be most vulnerable to global change since current environmental conditions predicted the assembly of these fungal communities. Climate change has already increased MAT by 1-3ºC in some boreal and arctic systems and this increase is expected to progress to 5 ºC by 2100 (Halim *et al*. 2018). These communities, and the EMF and saprotrophic components in particular, are likely to be the most responsive to climate change, and a shift in their relative abundances could have global implications for soil C cycling via feedbacks with tree growth and soil organic matter decomposition (Averill et al. 2014).

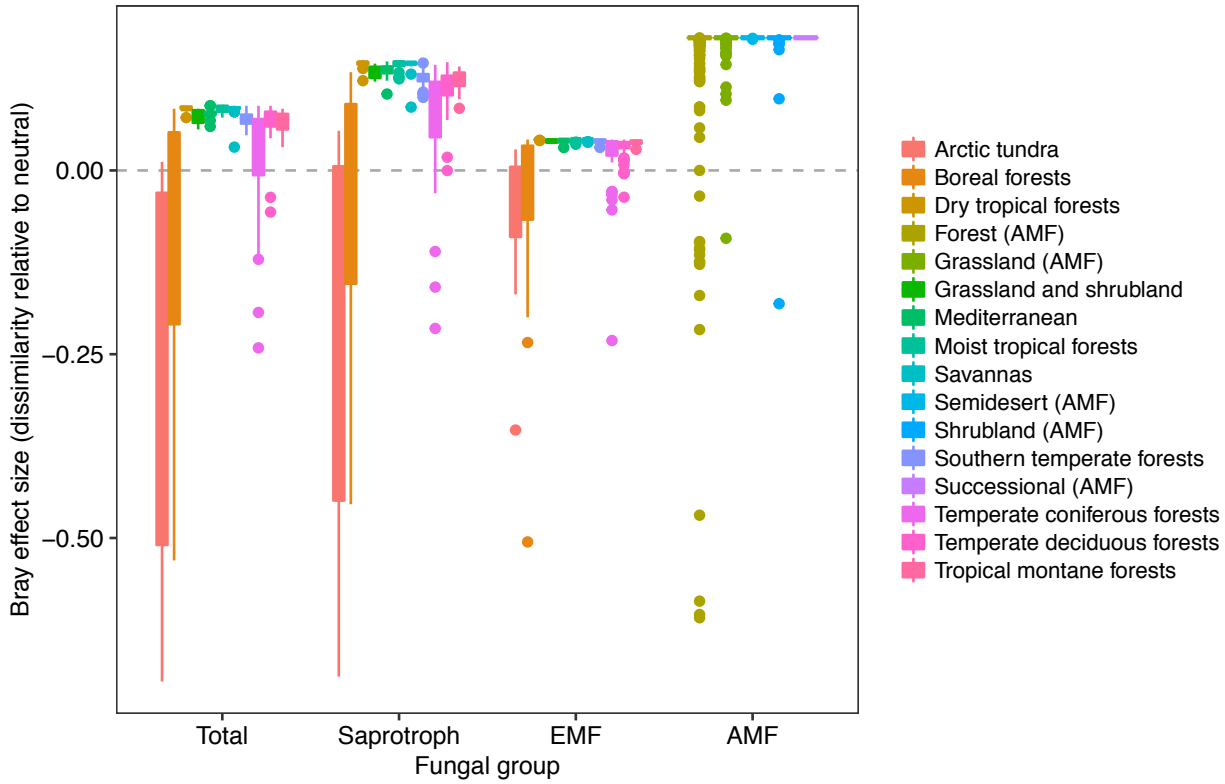


**Figure 4.1.** Effect sizes showing fungal community composition (Bray-Curtis) relative to a neutral model across biomes for the total fungal community and specific fungal functional groups.

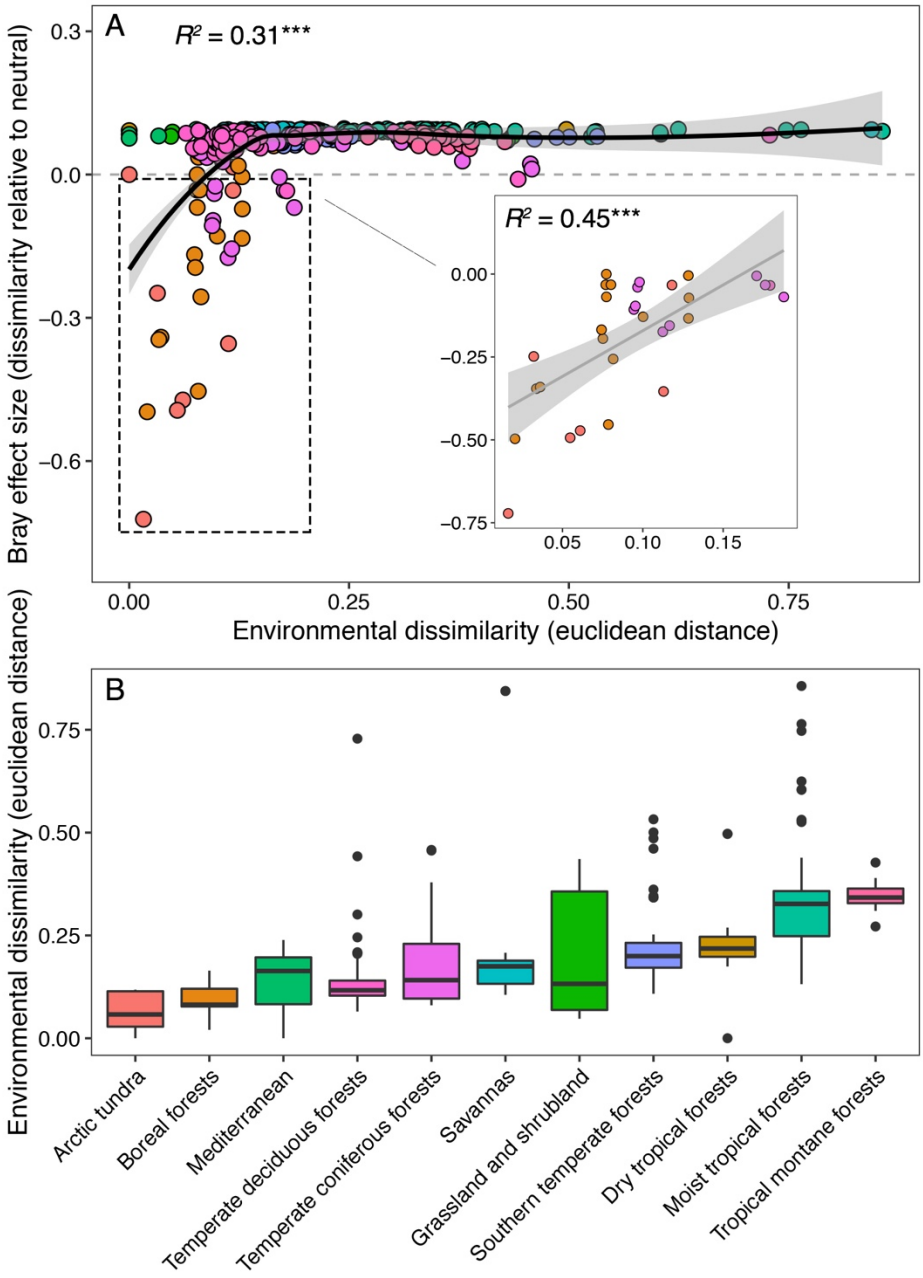


**Figure 4.2.** The relationship between effect size (Bray-Curtis) and environmental dissimilarity (A) and environmental dissimilarity across biomes (B). The insert in panel A shows the correlation for negative effect sizes included in the dashed area. Asterisks indicate significant correlations, where * = $P < 0.01$, ** = $P < 0.001$, *** = $P < 0.0001$, **** = $P < 0.00001$.

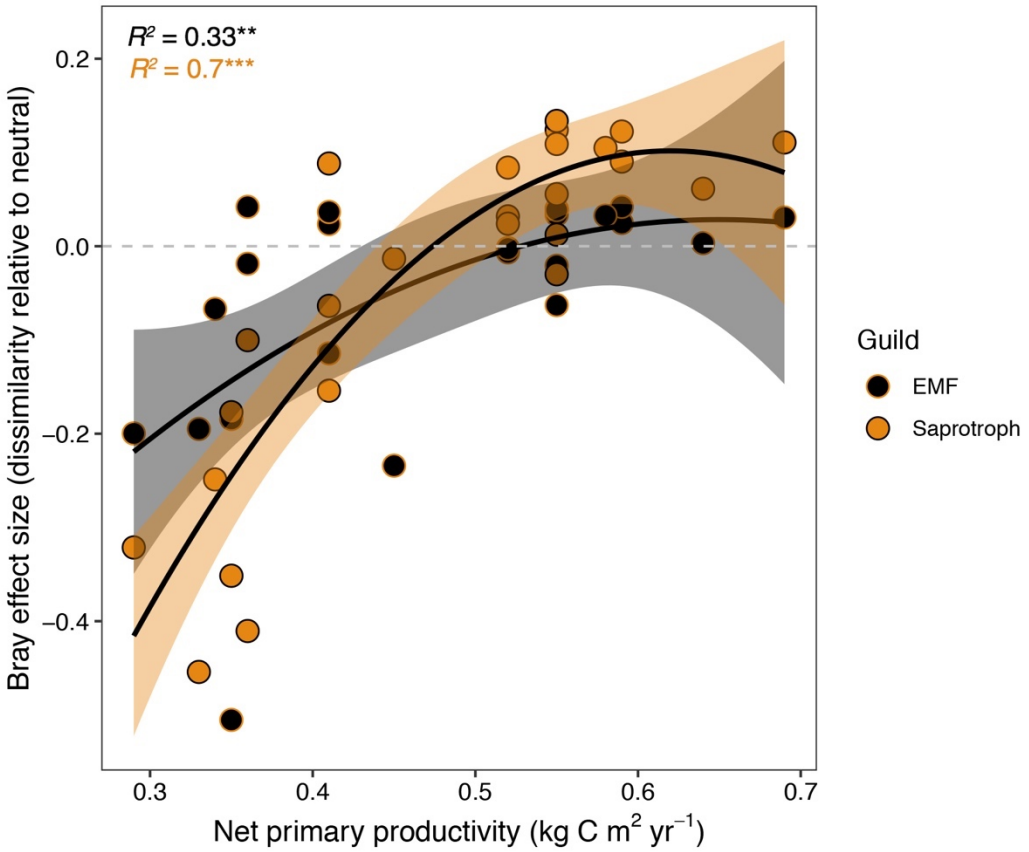


**Figure 4.3.** The correlation between effect size (Bray-Curtis) and net primary productivity in boreal forests comparing saprotrophs and EMF. Boreal forests were the only biome where this correlation was significant. Asterisks indicate significant correlations, where ** = $P < 0.001$, *** = $P < 0.0001$.

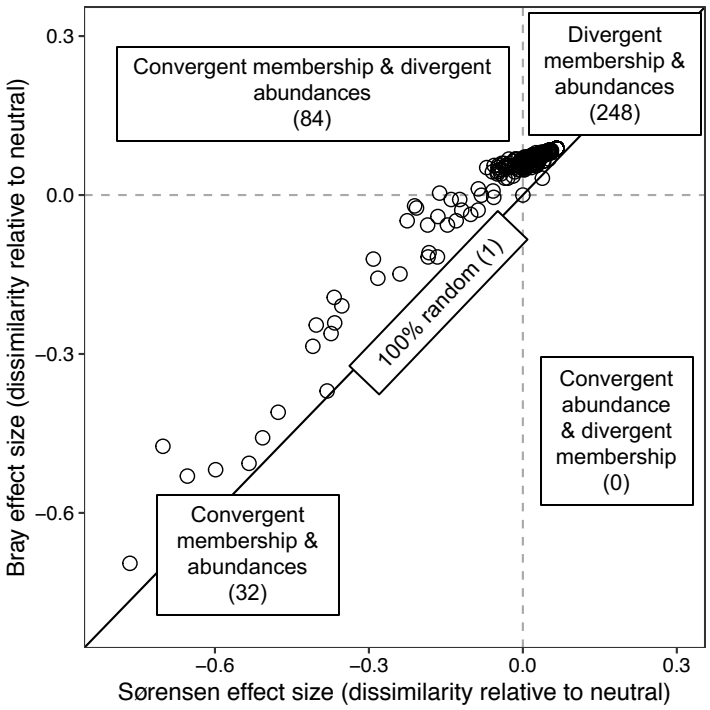


**Figure 4.4.** Effect sizes based on community dissimilarity using Bray-Curtis and Sørensen dissimilarities. Bray-Curtis and Sørensen are derived the same way, but Bray-Curtis is calculated based on the abundances of shared species, while Sørensen is calculated based on the number of shared species. A 1:1 line is plotted to show deviation between the two models. Different compartments are labelled to show where membership and abundance diverge and converge relative to a neutral model. Values in parentheses are the number of plots.

# CONCLUSION

The first two experiments in this dissertation addressed the impacts of garlic mustard invasions on soil fungi and edaphic properties. In chapter one, I found that both fungal communities and soil properties were altered in association with garlic mustard invasion and neither were responsive to three years of garlic mustard eradication. Managing invasive species for plant communities generally does not produce positive outcomes (35% of the time), so the lack of recovery observed in the fungal community is not surprising. However, the increase in ectomycorrhizal fungi in the eradicated plots and decrease in plant pathogens may help native tree seedlings recover from invasion, though it is unclear how sustained alterations to ectomycorrhizal community composition could affect tree seedling recovery. It is possible that long term garlic mustard eradications will eventually restore soil systems back to uninvaded, reference condition, but to my knowledge no study has examined long-term impacts of invasive plant eradications on soils. Soils in the eradicated plots could be altered for decades or longer, much like the human microbiome following a serious disturbance, but if eradicated areas are less prone to reinvasion and more likely to recruit native plants then this is a positive outcome.

The difficulty of restoring fungal communities following garlic mustard eradication is especially concerning since our second study found that soil warming amplified the impacts of garlic mustard invasion. If the impacts of garlic mustard invasion worsen because of climate warming, as indicated in the simulated soil warming experiment, then we should expect changes in the understory forest composition due to mycorrhizal fungal exclusion. Our study was in a single forest understory, so it is unclear how representative it is of broader garlic mustard invasions. However, in chapter one, fungal communities from a different series of garlic mustard

plots at the Harvard Forest converged to become similar to communities from other invaded plots across Southern New England, so the results of chapter two are likely informative of regional invasions. If other invasive species, in addition to garlic mustard, also have steeper impacts with warming then it will be important to increase stewardship efforts now in order to preemptively reduce invasive species loadings. This type of information may help stewardship offices access regional funding for garlic mustard management.

Understanding the mechanisms of plant-fungal interactions can lead to improved management in natural and agricultural systems. For example, there is a practice of using *Brassicas* to reduce fungal pathogen loads in agricultural systems, either as a biofumigant or cover crop. Part of the rationale for this practice is the presence of glucosinolates in *Brassica* tissues. Here, we show that glucosinolates also function to deter AMF on the model Brassicaceae, *Arabidopsis*. Presumably, Brassicaceae have been co-evolving with AMF longer than any particular fungal pathogen or herbivore because generalist AMF are found in nearly every soil (*e.g. Rhizophagus irregularis*). While we do not know the evolutionary history of glucosinolate production, I clearly show for the first time that glucosinolates play an important role in Brassicaceae growth and development by suppressing AMF. It would be interesting if future work examined the roles of other glucosinolates beside the indolic ones since Brassicaceae produce different quantities of indolic, aliphatic, and cyclic glucosinolates. It is possible that the ratio of indolic versus other types of glucosinolates influences resistance to AMF, and this would be important information for improving agricultural plant breeding and choosing crops that may be tolerant of AMF.

While my work has centered on garlic mustard or the Brassicaceae as a group, it has also focused on fungi, and the factors that influence fungal communities. My final chapter attempted

to disentangle different findings about the processes that structure soil fungi. While there is a role for environmental filtering in many studies on fungal communities, the relative importance of neutral processes is infrequently quantified, especially as a process that occurs simultaneously to environmental filtering. This chapter shows that at the largest spatial scale, and within biomes, fungi mostly assemble via stochastic processes. This does not negate earlier findings that show impacts of environmental variables on fungi across the globe, it just shows that stochastic processes are more dominant than environmental filtering. In northern latitude ecosystems the opposite pattern was observed. Niche assembly was found to be most important, especially for ectomycorrhizal fungi and saprotrophs. Arbuscular mycorrhizal fungi also assembled more via niche processes in northern latitude, cold temperature forests. Our results suggest that this could be due to the smaller height of plants in northern latitude forests compared to southern latitude ecosystems. Since the most dominant soil fungal guilds in northern latitude ecosystems (e.g. ectomycorrhizal fungi, saprotrophs) were primarily structured by current environmental conditions, changes in temperature, soil properties, and plant communities could more strongly impact fungi in the north than in mid and southern latitude ecosystems.

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# APPENDIX

**Supplementary Table 1.1.** Primer constructs designed for sequencing on Illumina MiSeq platforms and multiplexing using dual-barcoded *f*ITS7 and ITS4 primers

| Name | 5' Illumina adapter / RC 3' Illumina adapter | Index | Pad | Linker | PCR specific primer fITS7 / ITS4 |
|---|---|---|---|---|---|
| SC501_FITS7 | CAAGCAGAAGACGGCATACGAGAT | ACGACGTG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC502_FITS7 | CAAGCAGAAGACGGCATACGAGAT | ATATACAC | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC503_FITS7 | CAAGCAGAAGACGGCATACGAGAT | CGTCGCTA | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC504_FITS7 | CAAGCAGAAGACGGCATACGAGAT | CTAGAGCT | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC505_FITS7 | CAAGCAGAAGACGGCATACGAGAT | GCTCTAGT | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC506_FITS7 | CAAGCAGAAGACGGCATACGAGAT | GACACTGA | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC507_FITS7 | CAAGCAGAAGACGGCATACGAGAT | TGCGTACG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC508_FITS7 | CAAGCAGAAGACGGCATACGAGAT | TAGTGTAG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD501_FITS7 | CAAGCAGAAGACGGCATACGAGAT | AAGCAGCA | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD502_FITS7 | CAAGCAGAAGACGGCATACGAGAT | ACGCGTGA | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD503_FITS7 | CAAGCAGAAGACGGCATACGAGAT | CGATCTAC | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD504_FITS7 | CAAGCAGAAGACGGCATACGAGAT | TGCGTCAC | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD505_FITS7 | CAAGCAGAAGACGGCATACGAGAT | GTCTAGTG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD506_FITS7 | CAAGCAGAAGACGGCATACGAGAT | CTAGTATG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD507_FITS7 | CAAGCAGAAGACGGCATACGAGAT | GATAGCGT | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD508_FITS7 | CAAGCAGAAGACGGCATACGAGAT | TCTACACT | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD701_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | ACCTAGTA | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD702_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | ACGTACGT | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD703_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | ATATCGCG | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD704_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | CACGATAG | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD705_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | CGTATCGC | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD706_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | CTGCGACT | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD707_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | GCTGTAAC | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD708_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | GGACGTTA | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD710_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | TAAGTCTC | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD711_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | TACACAGT | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD712_ITS4 | AATGATACGGCGACCACCGAGATCT ACAC | TTGACGCA | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |

**Read 1 (fITS7) sequence primer (Tm = 65.8)**

TATGGTAATT GC TTGGAGGGCAAGTCTGGTGCC

**Read 2 (ITS4) sequence primer (Tm = 67.7)**

GGTCTGCGCG GA GAACCCAAACACTTTGGTTTCC

**Index (fITS7-ITS4) sequencing primer (Tm = 67.7)**

GGAAACCAAAGTGTTTGGGTTC TC CGCGCAGACC

**Supplementary Table 1.2.** Summary of sequence results, quality control, taxonomic identification and fungal guild annotation

| | **Total Sequences** | **After QC (# seqs)** | **Merging (# of merged seqs)** | **OTUs (#)** | **Taxonomy (# of OTUs)** | **Guild (# of OTUs)** |
|---|---|---|---|---|---|---|
| One year | 18,123,596 | 16,130,000 | 8,190,000 | 4,226 | 1,768 | 956 |
| Three years | 16,153,846 | 14,700,000 | 5,010,000 | 11,847 | 1,877 | 913 |

**Supplementary Table 3.** Linear effects mixed model results for soil physical and chemical properties, fungal biomass, and fungal richness.

| Year one | Soil pH | DF (btw) | DF (within) | F-value | P-value |
|---|---|---|---|---|---|
| | Site | 7 | 94 | 43.5 | < 0.0001 |
| | Invasion | 2 | 94 | 16.5 | < 0.0001 |
| | Horizon | 1 | 94 | 2.2 | 0.143 |
| | Site x Horizon | 14 | 94 | 2.7 | 0.002 |
| | Site x Horizon | 7 | 94 | 1.7 | 0.128 |
| | Invasion x Horizon | 2 | 94 | 2.2 | 0.118 |
| | Site x Invasion x Horizon | 14 | 94 | 0.5 | 0.951 |
| | **Ammonium** | | | | |
| | Site | 7 | 94 | 16.5 | < 0.0001 |
| | Invasion | 2 | 94 | 3.8 | 0.027 |
| | Horizon | 1 | 94 | 1.6 | 0.213 |
| | Site x Horizon | 14 | 94 | 1.6 | 0.083 |
| | Site x Horizon | 7 | 94 | 1.5 | 0.167 |
| | Invasion x Horizon | 2 | 94 | 0.4 | 0.705 |
| | Site x Invasion x Horizon | 14 | 94 | 0.9 | 0.574 |
| | **Nitrate** | | | | |
| | Site | 7 | 94 | 2.4 | 0.029 |
| | Invasion | 2 | 94 | 2.3 | 0.102 |
| | Horizon | 1 | 94 | 14.5 | < 0.0001 |
| | Site x Horizon | 14 | 94 | 0.4 | 0.966 |
| | Site x Horizon | 7 | 94 | 1.1 | 0.380 |
| | Invasion x Horizon | 2 | 94 | 3.5 | 0.035 |
| | Site x Invasion x Horizon | 14 | 94 | 0.5 | 0.940 |
| | **Soil C stock** | | | | |
| | Site | 7 | 84 | 8.9 | < 0.0001 |
| | Invasion | 2 | 84 | 0.4 | 0.696 |
| | Horizon | 1 | 84 | 0.2 | 0.675 |
| | Site x Horizon | 14 | 84 | 0.9 | 0.528 |
| | Site x Horizon | 7 | 84 | 1.9 | 0.073 |
| | Invasion x Horizon | 2 | 84 | 0.4 | 0.703 |
| | Site x Invasion x Horizon | 14 | 84 | 0.8 | 0.700 |
| | **Soil C:N ratio** | | | | |
| | Site | 7 | 84 | 3.1 | 0.006 |
| | Invasion | 2 | 84 | 0.7 | 0.503 |
| | Horizon | 1 | 84 | 5.6 | 0.020 |
| | Site x Horizon | 14 | 84 | 0.9 | 0.583 |
| | Site x Horizon | 7 | 84 | 0.7 | 0.655 |
| | Invasion x Horizon | 2 | 84 | 0.1 | 0.945 |
| | Site x Invasion x Horizon | 14 | 84 | 1.0 | 0.508 |
| | **Fungal biomass** | | | | |
| | Site | 7 | 94 | 5.5 | < 0.0001 |
| | Invasion | 2 | 94 | 4.6 | 0.013 |
| | Horizon | 1 | 94 | 49.7 | < 0.0001 |
| | Site x Horizon | 14 | 94 | 0.6 | 0.841 |
| | Site x Horizon | 7 | 94 | 1.4 | 0.214 |
| | Invasion x Horizon | 2 | 94 | 1.6 | 0.215 |
| | Site x Invasion x Horizon | 14 | 94 | 0.9 | 0.590 |
| | **Fungal richness** | | | | |
| | Site | 7 | 90 | 1.6 | 0.150 |
| | Invasion | 2 | 90 | 8.5 | 0.000 |
| | Horizon | 1 | 90 | 26.4 | < 0.0001 |
| | Site x Horizon | 14 | 90 | 2.9 | 0.001 |
| | Site x Horizon | 7 | 90 | 0.5 | 0.800 |
| | Invasion x Horizon | 2 | 90 | 0.4 | 0.700 |
| | Site x Invasion x Horizon | 14 | 90 | 0.9 | 0.500 |

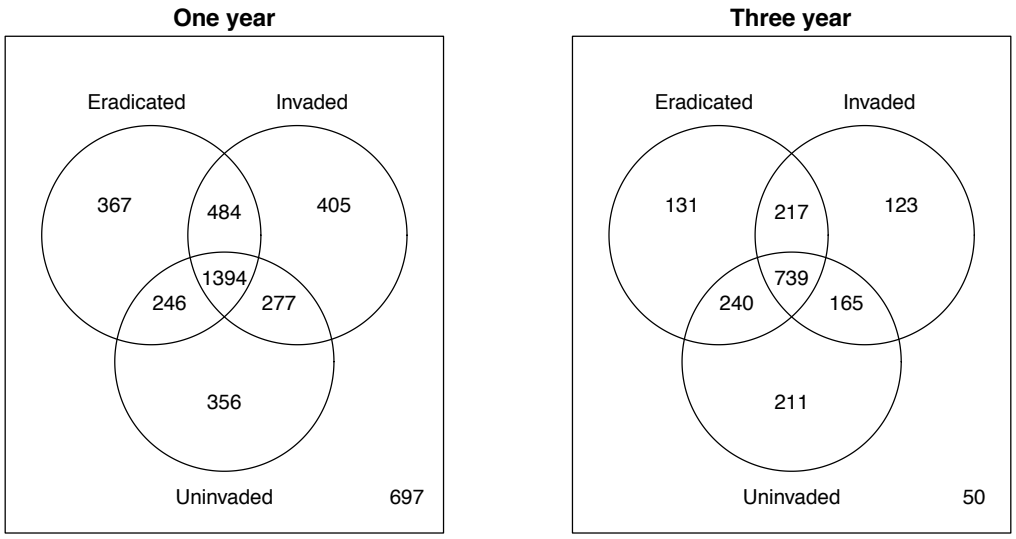
| | | | | | |
|---|---|---|---|---|---|
| **Year three** | **Soil pH** | | | | |
| | Site | 7 | 91 | 12.0 | < 0.0001 |
| | Invasion | 2 | 91 | 5.6 | 0.005 |
| | Horizon | 1 | 91 | 0.9 | 0.337 |
| | Site x Horizon | 14 | 91 | 1.8 | 0.055 |
| | Site x Horizon | 7 | 91 | 0.8 | 0.622 |
| | Invasion x Horizon | 2 | 91 | 0.1 | 0.926 |
| | Site x Invasion x Horizon | 14 | 91 | 0.6 | 0.836 |
| | **Ammonium** | | | | |
| | Site | 7 | 88 | 1.9 | 0.088 |
| | Invasion | 2 | 88 | 3.5 | 0.033 |
| | Horizon | 1 | 88 | 8.9 | 0.004 |
| | Site x Horizon | 14 | 88 | 1.1 | 0.389 |
| | Site x Horizon | 7 | 88 | 0.9 | 0.494 |
| | Invasion x Horizon | 2 | 88 | 0.3 | 0.752 |
| | Site x Invasion x Horizon | 14 | 88 | 1.1 | 0.393 |
| | **Nitrate** | | | | |
| | Site | 7 | 88 | 7.8 | < 0.0001 |
| | Invasion | 2 | 88 | 3.9 | 0.023 |
| | Horizon | 1 | 88 | 12.1 | 0.001 |
| | Site x Horizon | 14 | 88 | 1.0 | 0.421 |
| | Site x Horizon | 7 | 88 | 1.3 | 0.262 |
| | Invasion x Horizon | 2 | 88 | 3.7 | 0.028 |
| | Site x Invasion x Horizon | 14 | 88 | 0.7 | 0.733 |
| | **Soil C stock** | | | | |
| | Site | 7 | 78 | 53.2 | 0.000 |
| | Invasion | 2 | 78 | 3.3 | 0.041 |
| | Horizon | 1 | 78 | 2.3 | 0.130 |
| | Site x Horizon | 14 | 78 | 3.4 | 0.000 |
| | Site x Horizon | 7 | 78 | 2.2 | 0.040 |
| | Invasion x Horizon | 2 | 78 | 0.3 | 0.746 |
| | Site x Invasion x Horizon | 14 | 78 | 1.1 | 0.346 |
| | **Soil C:N ratio** | | | | |
| | Site | 7 | 78 | 7.5 | < 0.0001 |
| | Invasion | 2 | 78 | 8.4 | 0.000 |
| | Horizon | 1 | 78 | 5.9 | 0.018 |
| | Site x Horizon | 14 | 78 | 1.0 | 0.422 |
| | Site x Horizon | 7 | 78 | 0.9 | 0.543 |
| | Invasion x Horizon | 2 | 78 | 1.3 | 0.281 |
| | Site x Invasion x Horizon | 14 | 78 | 0.5 | 0.942 |
| | **Fungal biomass** | | | | |
| | Site | 7 | 91 | 11.4 | 0.000 |
| | Invasion | 2 | 91 | 0.5 | 0.614 |
| | Horizon | 1 | 91 | 131.4 | 0.000 |
| | Site x Horizon | 14 | 91 | 0.4 | 0.972 |
| | Site x Horizon | 7 | 91 | 2.4 | 0.024 |
| | Invasion x Horizon | 2 | 91 | 0.1 | 0.889 |
| | Site x Invasion x Horizon | 14 | 91 | 0.7 | 0.755 |
| | **Fungal richness** | | | | |
| | Site | 7 | 82 | 1.5 | 0.160 |
| | Invasion | 2 | 82 | 0.9 | 0.400 |
| | Horizon | 1 | 82 | 0.0 | 0.900 |
| | Site x Horizon | 14 | 82 | 1.3 | 0.200 |
| | Site x Horizon | 7 | 82 | 0.6 | 7.000 |
| | Invasion x Horizon | 2 | 82 | 0.5 | 0.600 |
| | Site x Invasion x Horizon | 14 | 82 | 0.7 | 0.700 |

**Supplementary Table 1.4.** Species (OTU) richness across invasion statuses and years after garlic mustard eradication. Values represent the mean and the standard error is in parentheses.

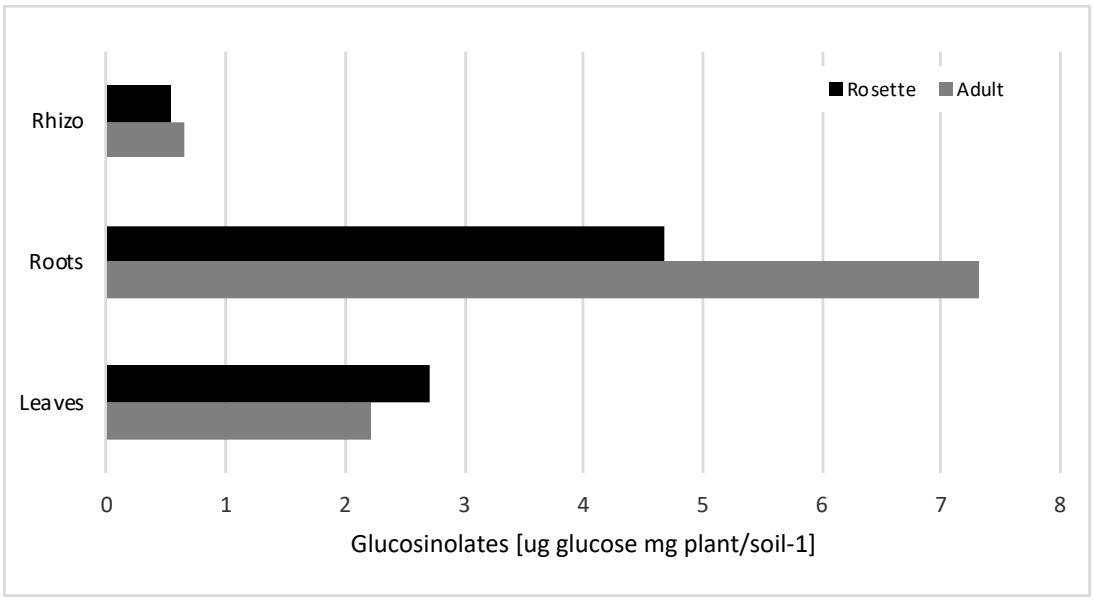
| | | **Richness (# of OTUS)** |
|---|---|---|
| One year | | |
| | Uninvaded | 183 (10) |
| | Invaded | 224 (8) |
| | Eradicated | 211 (7) |
| Three years | | |
| | Uninvaded | 88 (5) |
| | Invaded | 93 (4) |
| | Eradicated | 104 (6) |

**Supplementary Table 1.5.** The relative abundance of ectomycorrhizal Agaricales after one year of garlic mustard eradication. Values are the mean the stand error is in parentheses.

| | **Uninvaded** | **Invaded** | **Eradicated** |
|---|---|---|---|
| Amanita | 0.00064 (0.00009) | 0.00004 (0.00002) | **0.00096 (0.00051)** |
| Cortinarius | 0.00028 (0.00004) | 0.00002 (0.00001) | 0.00003 (0.00001) |
| Entoloma | 0.00006 (0.00001) | 0.00006 (0.00002) | 0.00006 (0.00001) |
| Gliophorus | 0.00071 (0.0001) | 0.00003 (0.00001) | **0.0013 (0.00097)** |
| Hebeloma | 0 (0) | 0 (0) | **0.00042 (0.0004)** |
| Hygrophorus | 0.00272 (0.00039) | 0.00001 (0.00001) | 0.00002 (0.00002) |
| Hymenogaster | 0.00004 (0.00001) | 0.00001 (0.00001) | **0.00822 (0.00664)** |
| Inocybe | 0.00049 (0.00007) | 0.00134 (0.00066) | **0.00214 (0.00095)** |
| Laccaria | 0.0027 (0.00039) | 0.00004 (0.00002) | 0.00041 (0.00028) |




**Supplementary Figure 1.1.** The number of shared fungal species (OTUs) between uninvaded, invaded, and eradicated plots after one and three years of garlic mustard removal. While patterns across invasion statuses should be compared across years, direct evaluation of OTU values between one and three years should not be compared since there were more sequences per sample (deeper sequencing depth) in the one year analysis compared to the three year analysis.




**Supplementary Figure 1.2.** Garlic mustard glucosinolate concentrations in rhizosphere soil (Rhizo), roots, and leaves comparing first year (Rosette) and second year (Adult) plants. Bars represent the mean of three replicates. Garlic mustard plants were harvested from an invaded patch in Durham, NH in June, 2014.

**Supplementary Table 2.1.** Primer constructs designed for sequencing on Illumina MiSeq platforms and multiplexing using dual-barcoded *f*ITS7/NS31 and ITS4/AML2 primers

| Name | 5' Illumina adapter / RC 3' Illumina adapter | Index | Pad | Linker | PCR specific primer FITS7 or NS31 / ITS4 or AML2 |
|---|---|---|---|---|---|
| SC501_FITS7 | CAAGCAGAAGACGGCATACGAGAT | ACGACGTG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC502_FITS7 | CAAGCAGAAGACGGCATACGAGAT | ATATACAC | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC503_FITS7 | CAAGCAGAAGACGGCATACGAGAT | CGTCGCTA | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC504_FITS7 | CAAGCAGAAGACGGCATACGAGAT | CTAGAGCT | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC505_FITS7 | CAAGCAGAAGACGGCATACGAGAT | GCTCTAGT | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC506_FITS7 | CAAGCAGAAGACGGCATACGAGAT | GACACTGA | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC507_FITS7 | CAAGCAGAAGACGGCATACGAGAT | TGCGTACG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SC508_FITS7 | CAAGCAGAAGACGGCATACGAGAT | TAGTGTAG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD501_FITS7 | CAAGCAGAAGACGGCATACGAGAT | AAGCAGCA | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD502_FITS7 | CAAGCAGAAGACGGCATACGAGAT | ACGCGTGA | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD503_FITS7 | CAAGCAGAAGACGGCATACGAGAT | CGATCTAC | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD504_FITS7 | CAAGCAGAAGACGGCATACGAGAT | TGCGTCAC | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD505_FITS7 | CAAGCAGAAGACGGCATACGAGAT | GTCTAGTG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD506_FITS7 | CAAGCAGAAGACGGCATACGAGAT | CTAGTATG | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD507_FITS7 | CAAGCAGAAGACGGCATACGAGAT | GATAGCGT | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD508_FITS7 | CAAGCAGAAGACGGCATACGAGAT | TCTACACT | GCAGCGAGCC | GG | GTGARTCATCGAATCTTTG |
| SD701_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | ACCTAGTA | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD702_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | ACGTACGT | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD703_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | ATATCGCG | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD704_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | CACGATAG | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD705_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | CGTATCGC | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD706_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | CTGCGACT | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD707_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | GCTGTAAC | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD708_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | GGACGTTA | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD710_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | TAAGTCTC | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD711_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | TACACAGT | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| SD712_ITS4 | AATGATACGGCGACCACCGAGATCTACAC | TTGACGCA | GGTCTGCGCG | AA | TCCTCCGCTTATTGATATGC |
| NS31_SA1 | CAAGCAGAAGACGGCATACGAGAT | ATCGTACG | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SA2 | CAAGCAGAAGACGGCATACGAGAT | ACTATCTG | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SA3 | CAAGCAGAAGACGGCATACGAGAT | TAGCGAGT | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SA4 | CAAGCAGAAGACGGCATACGAGAT | CTGCGTGT | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SB1 | CAAGCAGAAGACGGCATACGAGAT | CTACTATA | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SB2 | CAAGCAGAAGACGGCATACGAGAT | CGTTACTA | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SB3 | CAAGCAGAAGACGGCATACGAGAT | AGAGTCAC | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SB4 | CAAGCAGAAGACGGCATACGAGAT | TACGAGAC | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SC1 | CAAGCAGAAGACGGCATACGAGAT | ACGACGTG | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SC2 | CAAGCAGAAGACGGCATACGAGAT | ATATACAC | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SC3 | CAAGCAGAAGACGGCATACGAGAT | CGTCGCTA | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |

| | | | | | |
|---|---|---|---|---|---|
| NS31_SC4 | CAAGCAGAAGACGGCATACGAGAT | CTAGAGCT | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SD1 | CAAGCAGAAGACGGCATACGAGAT | AAGCAGCA | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SD2 | CAAGCAGAAGACGGCATACGAGAT | ACGCGTGA | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SD3 | CAAGCAGAAGACGGCATACGAGAT | CGATCTAC | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| NS31_SD4 | CAAGCAGAAGACGGCATACGAGAT | TGCGTCAC | TATGGTAATT | GC | TTGGAGGGCAAGTCTGGTGCC |
| AML2_SA1 | AATGATACGGCGACCACCGAGATCTACAC | AACTCTCG | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SA2 | AATGATACGGCGACCACCGAGATCTACAC | ACTATGTC | GGTCTGCGC | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SA3 | AATGATACGGCGACCACCGAGATCTACAC | AGTAGCGT | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SA4 | AATGATACGGCGACCACCGAGATCTACAC | CAGTGAGT | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SB1 | AATGATACGGCGACCACCGAGATCTACAC | AAGTCGAG | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SB2 | AATGATACGGCGACCACCGAGATCTACAC | ATACTTCG | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SB3 | AATGATACGGCGACCACCGAGATCTACAC | AGCTGCTA | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SB4 | AATGATACGGCGACCACCGAGATCTACAC | CATAGAGA | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SC1 | AATGATACGGCGACCACCGAGATCTACAC | ACCTACTG | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SC2 | AATGATACGGCGACCACCGAGATCTACAC | AGCGCTAT | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SC3 | AATGATACGGCGACCACCGAGATCTACAC | AGTCTAGA | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SC4 | AATGATACGGCGACCACCGAGATCTACAC | CATGAGGA | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SD1 | AATGATACGGCGACCACCGAGATCTACAC | ACCTAGTA | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SD2 | AATGATACGGCGACCACCGAGATCTACAC | ACGTACGT | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SD3 | AATGATACGGCGACCACCGAGATCTACAC | ATATCGCG | GGTCTGCGCG | GA | GAACCCAAACACTTTGGTTTCC |
| AML2_SD4 | AATGATACGGCGACCACCGAGATCTACAC | CACGATAG | GTCTGCGC | GA | GAACCCAAACACTTTGGTTTCC |

**Read 1 (fITS7) sequence primer (Tm = 65.8)**
TATGGTAATT GC TTGGAGGGCAAGTCTGGTGCC
**Read 2 (ITS4) sequence primer (Tm = 67.7)**
GGTCTGCGCG GA GAACCCAAACACTTTGGTTTCC
**Index (fITS7-ITS4) sequencing primer (Tm = 67.7)**
GGAAACCAAAGTGTTTGGGTTC TC CGCGCAGACC
**Read 1 (NS31) sequencing primer (Tm = 66.1)**
GCAGCGAGCCGGGTGARTCATCGAATCTTTG
**Read 2 (AML2) sequencing primer (Tm = 64.9)**
GGTCTGCGCGAATCCTCCGCTTATTGATATGC
**Index (NS31-AML2) sequencing primer (Tm = 66.3)**
GCATATCAATAAGCGGAGGATTCGCGCAGACC

**Supplementary Table 2.2.** Summary of bioinformatic sequence analyses, including sequence retention across steps and taxonomic and functional guild annotations to sequences.

| Marker | Total[1] | Trimming/ Merging[1] | ITSx[1] | Retention (%) | OTUs (#) | Non-fungal (ITS2) / non-AMF (18S) | Rarification level[1] | Taxonomy (% of OTUs) | Guild (% of OTUs) | Taxonomy (% of reads) | Guild (% of reads) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *ITS2* | 16,564,337 | 5,000,099 | 4,996,820 | 60.30% | 2011 | 8 | 1740 | 64% | 32% | 99% | 74% |
| *18S* | 24,000,000 | 7,440,000 | N.A. | 62% | 128 | 21 | 5056 | 84.40% | N.A. | 71% | N.A. |

[1] # of sequences

**Supplementary Table 2.3.** Table of statistical values for soil properties, processes, and fungal biomass. Values represent F-values and P-values (*F*-value;*P*-value) and significant effect are bolded.

| | Soil moisture (g $H_2O$ $g^{-1}$ soil) | Soil pH | Total soil C (g C $m^2$) | Inorganic N (ug N $g^{-1}$ dry soil) | N-min[1] (ug N $g^{-1}$ dry soil $day^{-1}$) | C-min[2] (mg $CO_2$ $g^{-1}$ dry soil $day^{-1}$) | Fine root biomass (mg dry roots $m^2$) | AMF biomass (nmol 16:1ω5c NLFA $g^{-1}$ dry soil) | Fungal biomass (nmol fungal PLFAs $g^{-1}$ dry soil) |
|---|---|---|---|---|---|---|---|---|---|
| Treatment* | 2.4; 0.08 | 2; 0.1 | **3.8; 0.01** | 0.4; 0.7 | 2.2; 0.1 | **4.2; 0.01** | 1.7; 0.2 | 1.4; 0.3 | **2.7; 0.055** |
| Invasion | 1.3; 0.3 | 0.2; 0.7 | 0.2; 0.7 | 1.3; 0.3 | 0.7; 0.4 | 0.3; 0.6 | 0.9; 0.4 | **4.1; 0.05** | 0.8; 0.4 |
| Horizon | **4.6; 0.04** | 1.6; 0.2 | **33.7; <0.001** | **13.1; 0.006** | **7.3; 0.009** | **45; <0.0001** | **16.3; 0.003** | **79; <0.001** | **120; <0.001** |
| Treatment × Invasion | 0.9; 0.5 | 0.7; 0.6 | 0.5; 0.7 | 1.4; 0.3 | 2; 0.1 | 0.2; 0.9 | **4.8; 0.007** | **10.2; <0.001** | 1.3; 0.3 |
| Treatment × Horizon | 1.3; 0.3 | 0.1; 1 | 0.1; 90.9 | 0.7; 0.6 | 0.6; 0.6 | 0.9; 0.4 | 0.1; 0.9 | 1.4; 0.3 | **5.4; 0.002** |
| Invasion × Horizon | 0.04; 0.9 | 0.1; 0.8 | 1.2; 0.3 | 0.2; 0.7 | 0.3; 0.6 | 0.3; 0.6 | 1.1; 0.3 | **5.3; 0.03** | 0.4; 0.6 |
| Treatment × Invasion × Horizon | 0.7; 0.6 | 0.2; 0.9 | 0.3; 0.9 | 1.3; 0.3 | 0.5; 0.7 | 0.4; 0.8 | 0.8; 0.5 | 2; 0.1 | 2.6; 0.06 |

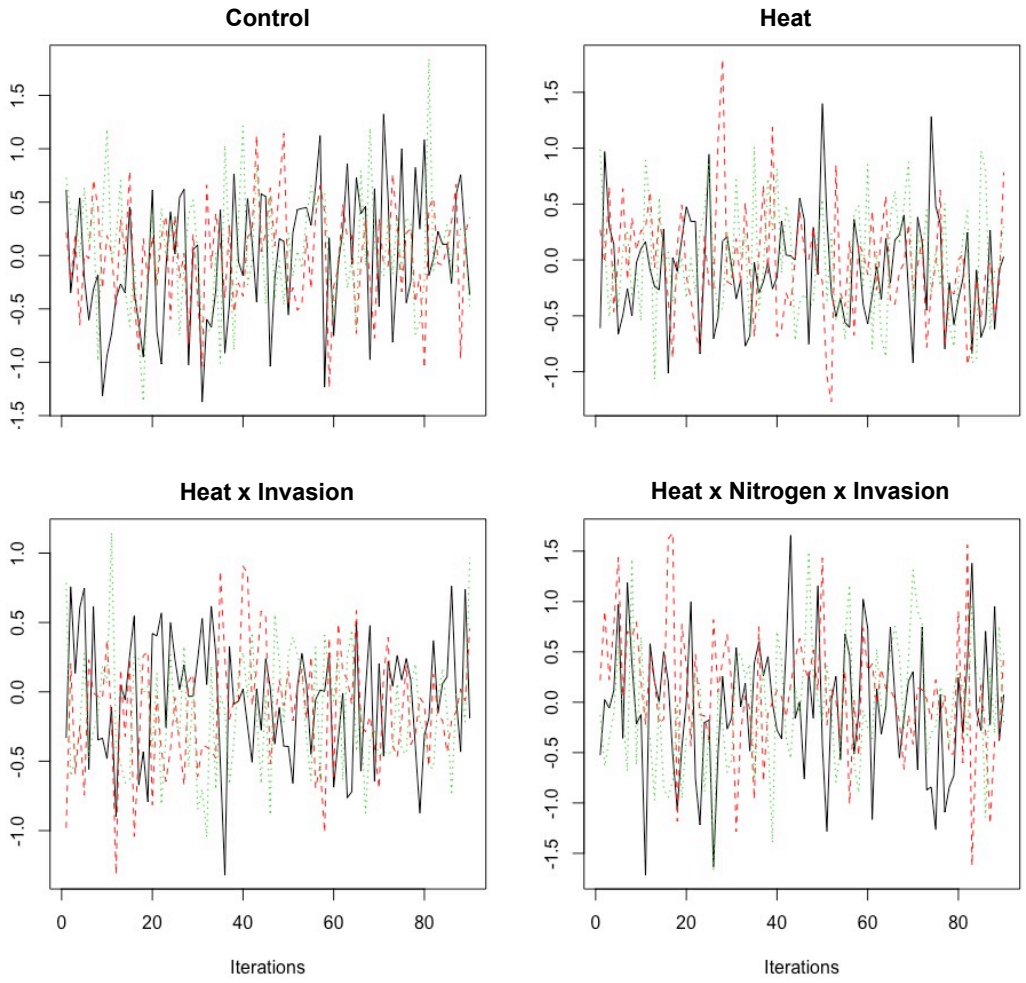
* Refers to the abiotic global change treatments

[1] Net soil N-mineralization is a measure of N-availability measured during a seven-day laboratory incubation.

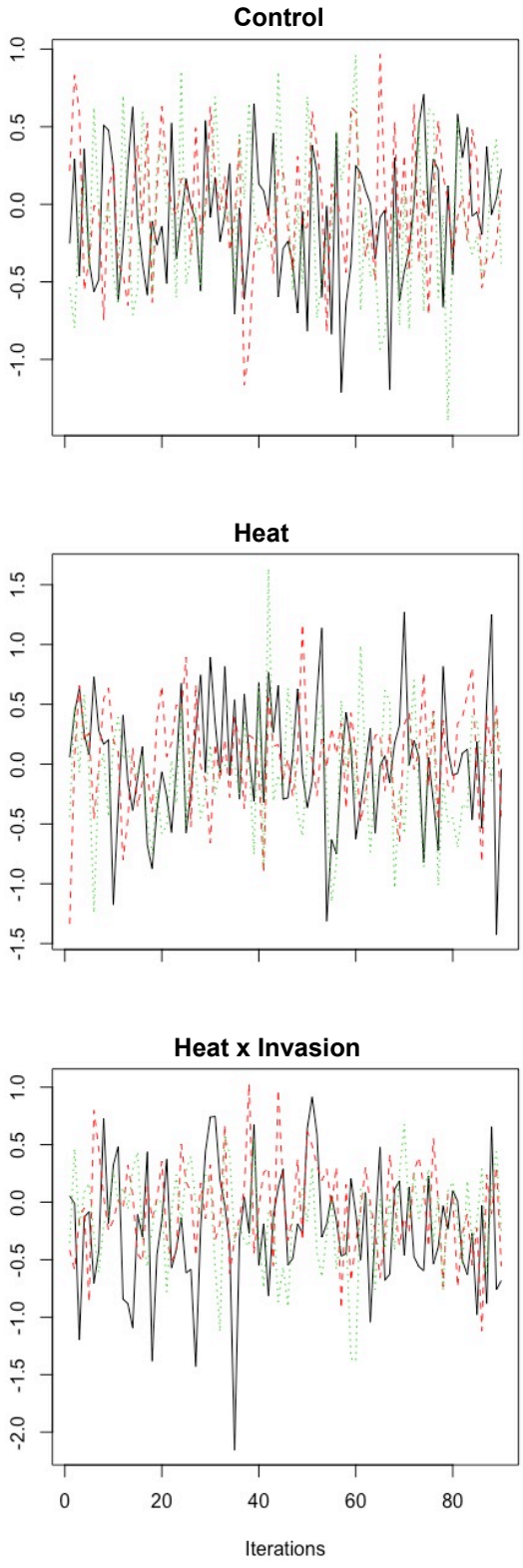
[2] C mineralization is a proxy for labile C availability measured during a ten-day laboratory incubation.

**Supplementary Table 2.4.** Table of statistical values for fungal community Bray-Curtis dissimilarity. Significance was determined using PERMANOVA and significant effects are in bold.

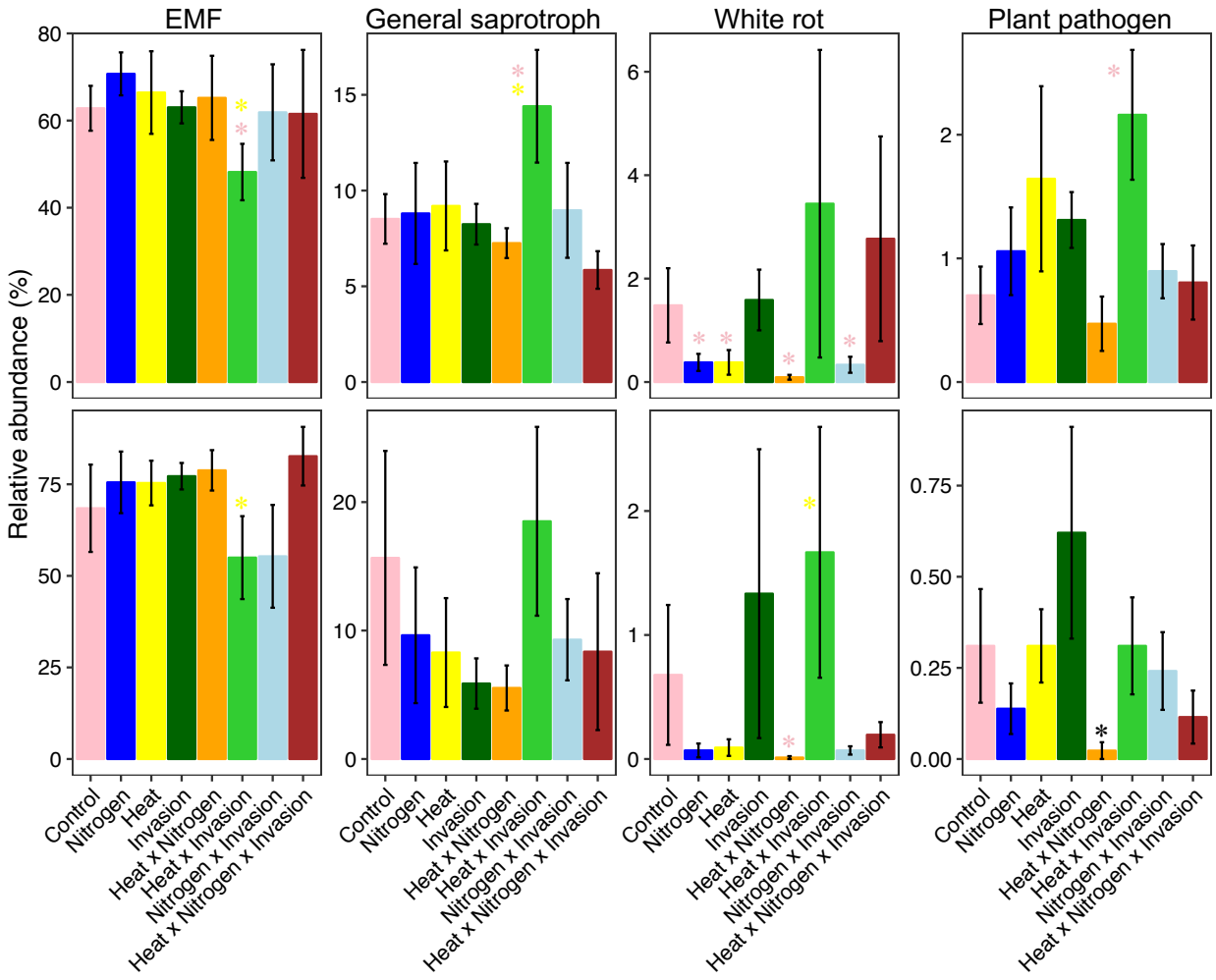
| | Fungi | | | AMF | | |
|---|---|---|---|---|---|---|
| Effect | Pseudo *F*-value | $R^2$ | *P*-value | Pseudo *F*-value | $R^2$ | *P*-value |
| Treatment | **1.72** | **0.06** | **0.002** | **1.86** | **0.07** | **0.006** |
| Invasion | 0.93 | 0.01 | 0.53 | **2.24** | **0.03** | **0.034** |
| Horizon | **2.75** | **0.03** | **0.001** | **7.01** | **0.09** | **0.001** |
| Treatment × Invasion | **0.49** | **0.05** | **0.041** | **1.65** | **0.06** | **0.03** |
| Treatment × Horizon | 0.79 | 0.03 | 0.9 | 0.63 | 0.02 | 0.95 |
| Invasion × Horizon | 0.25 | 0.01 | 0.9 | 0.39 | 0.01 | 0.06 |
| Treatment × Invasion × Horizon | 0.76 | 0.02 | 1.0 | 0.48 | 0.04 | 0.48 |




**Supplementary Figure 2.1.** Trace plots showing model mixing for three JSDM models of the ITS2 (Fungi) data.

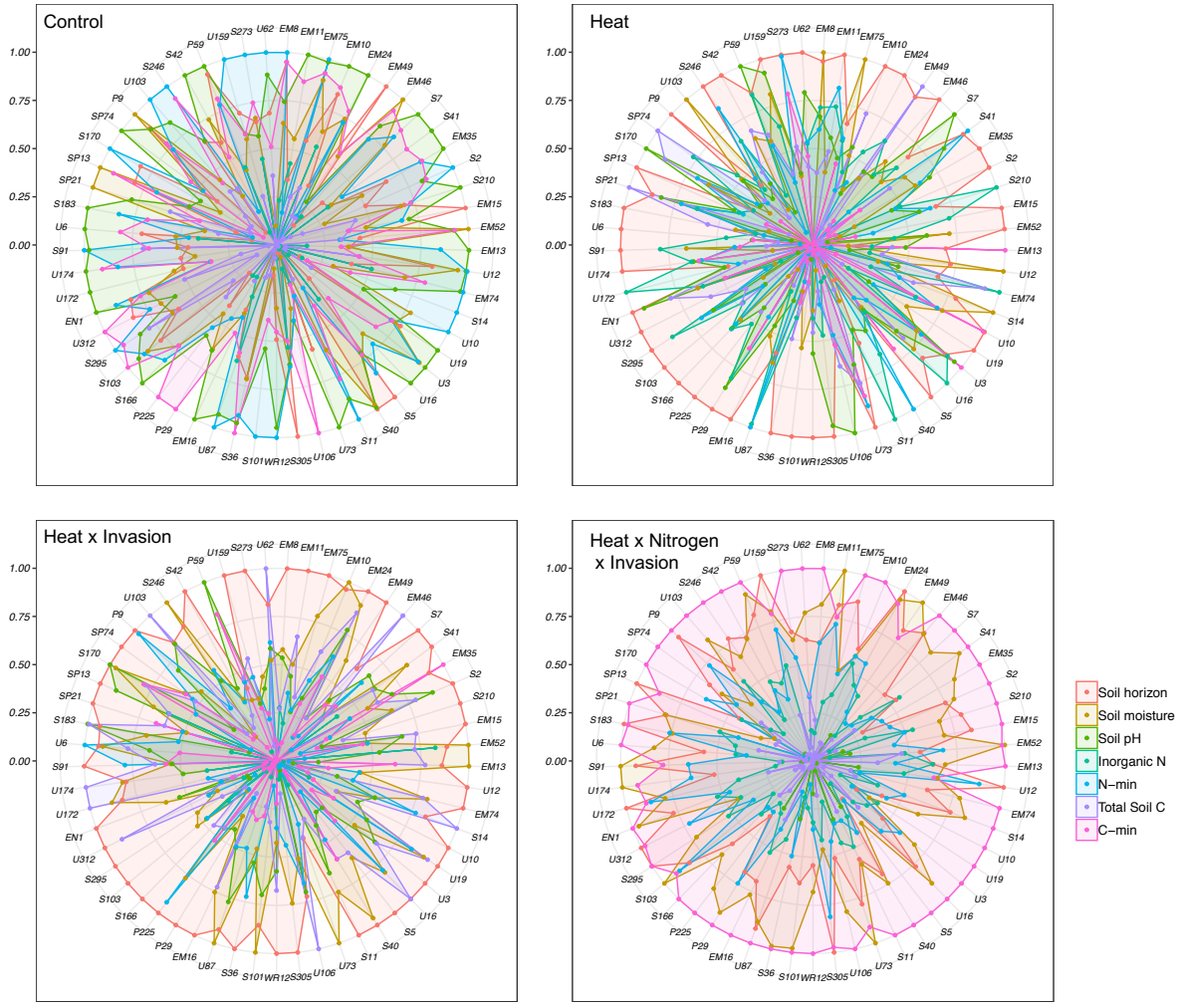



**Supplementary Figure 2.2.** Trace plots showing model mixing for three JSDM models of the 18S (AMF) data.

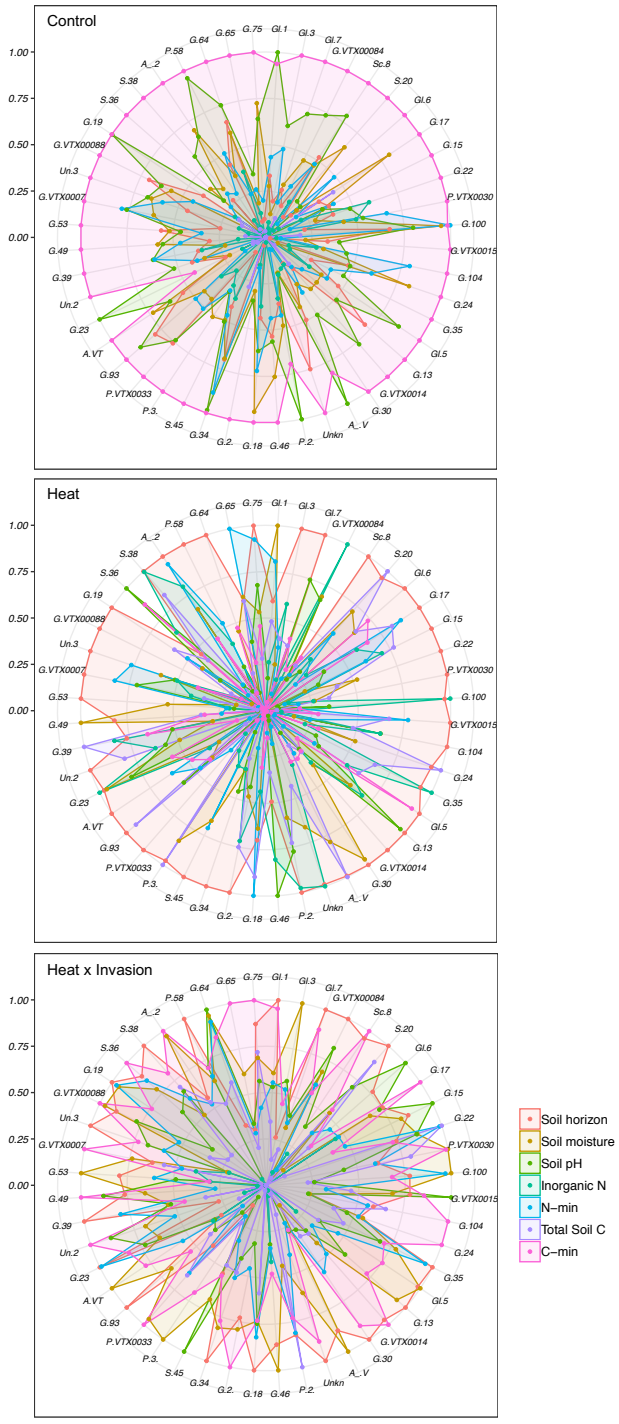



**Supplementary Figure 2.3.** The relative abundance of key functional guilds in all treatment plots in the organic (top) and mineral (bottom) soil. Bars represent the mean and error bars are the standard error. Asterisks indicate where differences were significant relative to the control (pink asterisks) or the soil warming plots (yellow asterisks).

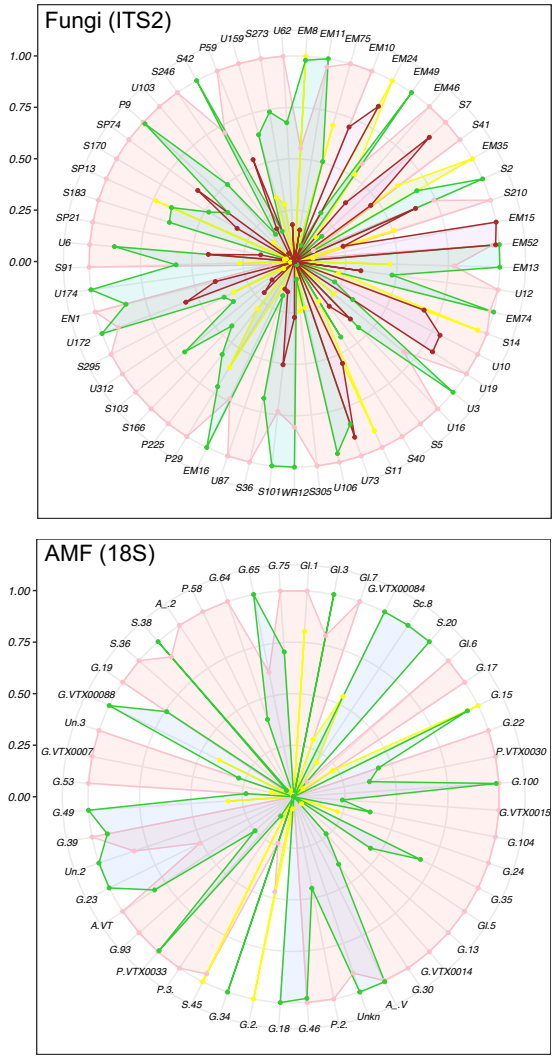
# Environmental Variation for Fungi (ITS2)



# Environmental Variation for AMF (18S)



**Random variation**



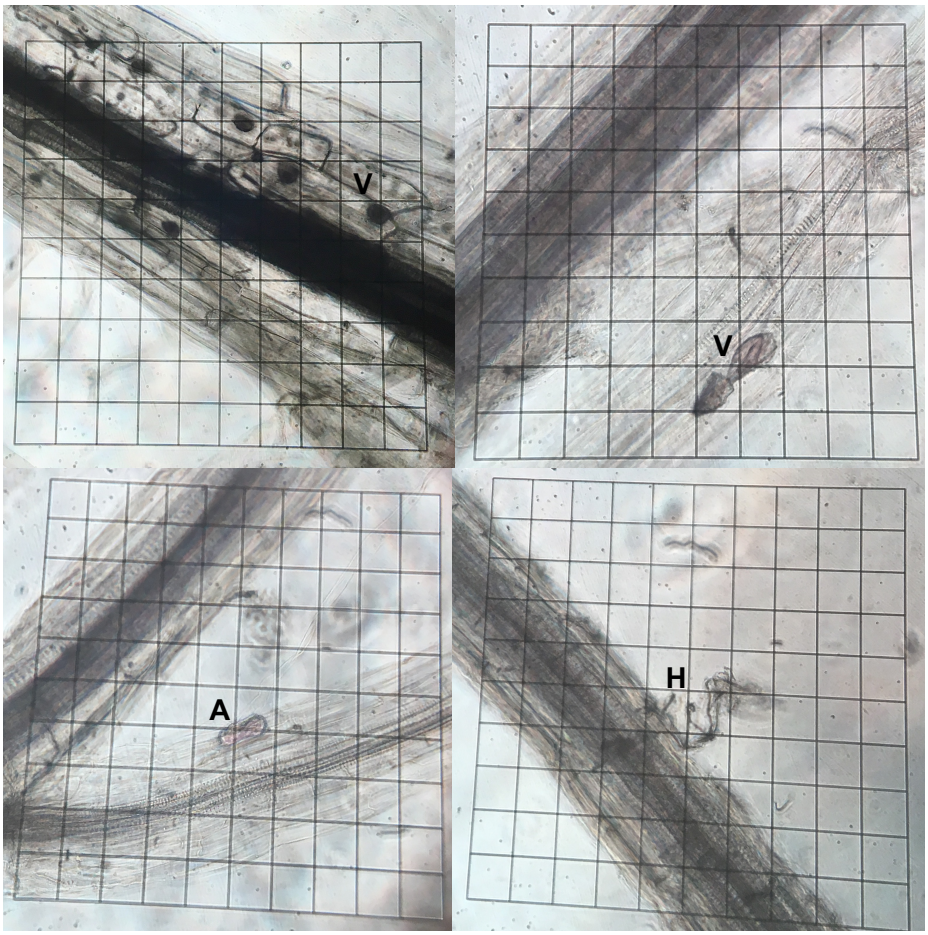

**Supplementary Figure 2.4**. The influence of soil properties and processes on fungal relative abundances and random variation in fungal relative abundances. Values represent the amount of variation explained by each variable after variance partioning and hierarchical joint species distribution modeling for the most common fungal taxa. Each position on the axis is a fungal taxon. For fungi, this includes EMF (EM), an endophyte (EN), pathogens (PP), undefined saprotrophs (S), undefined saprotroph-pathotrophs (SP), fungi with unknown guild membership (U), and a white rot fungus (WR). Fungal taxa are arranged in rank abundance with EM8 most abundant and U62 least abundant. Moving clockwise starting at EM8, taxon abbrevations are short for, Russula, Russula, Russula, Russula, Russula, Lactarius_camphoratus, Russula, Mortierellaceae, Umbelopsis, Cortinarius, Mortierellaceae, Umbelopsis, Cortinarius, Lactarius, Amanita, Leucosporidiales, Russula, Mortierella, Tremellomycetes, Chaetothyriales, Fungus, Mortierellomycota, Mortierella, Umbelopsis, Saccharomycetales, Fungus, Chaetothyriales, Saccharomycetales, Mycena, Mortierella, Mortierella, Solicoccozyma_terricola, Tricholoma, Cryptococcus, Cladophialophora, Mortierella_horticola, Megacollybia, Chytridiomycota, Ramicandelaber_taiwanensis, Chaetothyriales, Trichoderma_asperellum, Fungus, Saccharomycetales, Endogonales, Talaromyces, Xenasmatella, Herpotrichiellaceae, Mortierella, Trichoderma, Cladophialophora, Tremellomycetes, Mortierella, Ramicandelaber_taiwanensis, Verticillium_leptobactrum, Chytridiomycota, Clavariaceae, Unknown. AMF taxa are arranged in rank abundance with GL.1 most abundant and G.75 least abundant. Moving clockwise starting at GL.1, taxon abbreviations are short for, *Glomus 1, Glomus 3, Glomus 7, Glomus VTX00084, Scutellospora 8, Scutellospora 20, Glomus 6, Glomus 17, Glomus 15, Glomus 22, Paraglomus VTX00308, Glomus 100, Glomus VTX00151, Glomus 104, Glomus 24, Glomus 35, Glomus 5, Glomus 13, Glomus VTX00149, Glomus 30, Ambispora_leptoticha VTX00242, Unknown, Paraglomus 2 VTX00308, Glomus 46, Glomus 18, Glomus 2 VTX00084, Glomus 34, Scutellospora 45, Paraglomus 3 VTX00308, Paraglomus VTX00336, Glomus 93,. Archaeospora VTX00009, Glomus 23, Unknown 2, Glomus 39, Glomus 49, Glomus 53, Glomus VTX00070, Unknown 3, Glomus VTX00088, Glomus 19, Scutellospora 36, Scutellospora 38, Ambispora_leptoticha 2 VTX00242, Paraglomus 58, Glomus 64, Glomus 65, Glomus 75.*

**Supplementary Table 3.1.** Mixed effects model outputs comparing the effects of genotype (IG-removed, wild-type, IG-enhanced), AMF addition level (0, 10, and 50 spores $g^{-1}$ dry soil), and their interaction.

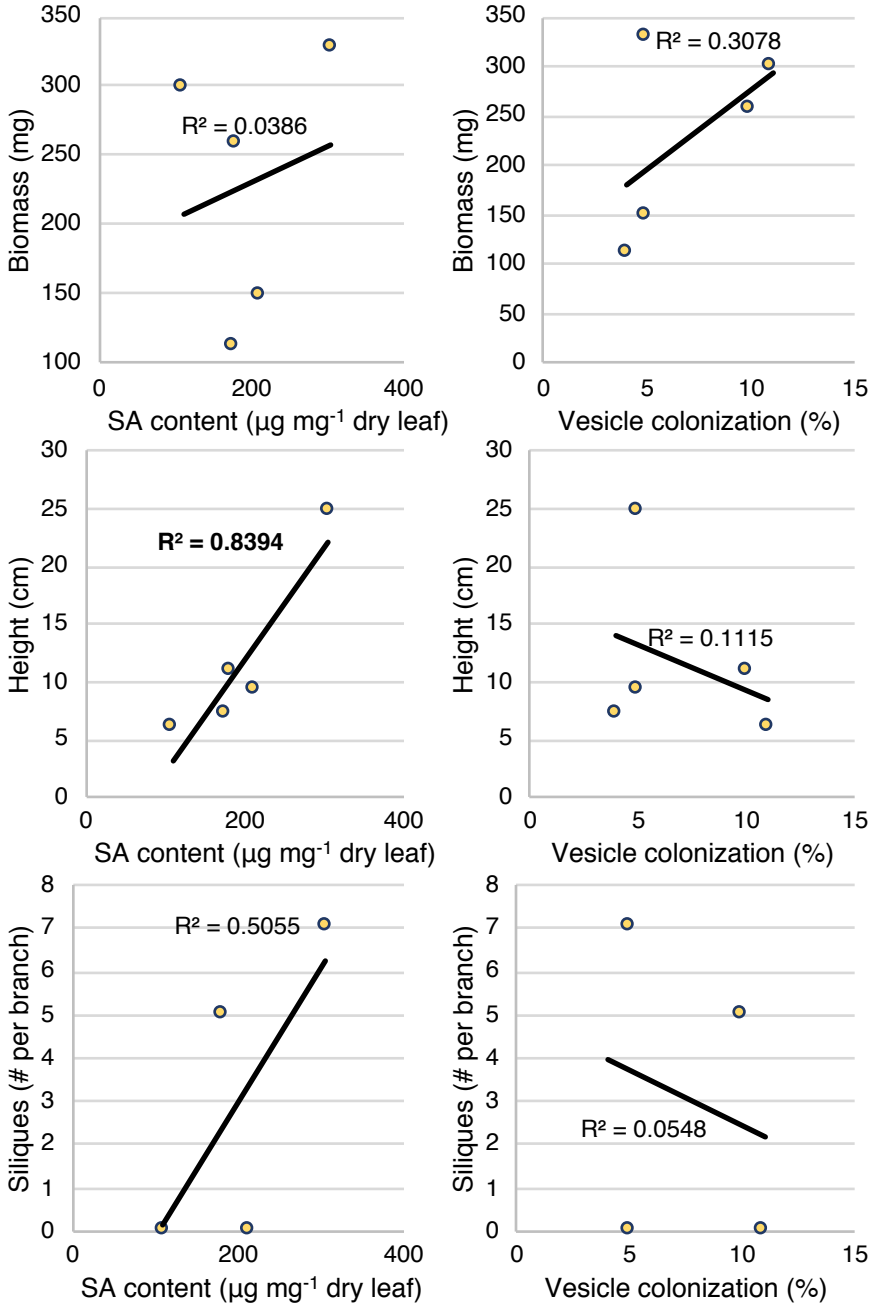
| | Genotype | AMF addition | Genotype × AMF addition |
|---|---|---|---|
| **Overall performance** | $F_{(2,38)} = 1087$; $P < 0.001$ | $F_{(1,38)} = 3.8$; $P = 0.06$ | $F_{(2,38)} = 35$; $P < 0.001$ |
| **Reproductive output** | $F_{(2,38)} = 160$; $P < 0.001$ | $F_{(1,38)} = 5.3$; $P = 0.03$ | $F_{(2,38)} = 4.26$; $P = 0.02$ |
| **Height** | $F_{(2,38)} = 69$; $P < 0.001$ | $F_{(1,38)} = 0.87$; $P = 0.4$ | $F_{(2,38)} = 11$; $P = 0.002$ |
| **Aboveground biomass** | $F_{(2,38)} = 34$; $P < 0.001$ | $F_{(1,38)} = 16$; $P = 0.003$ | $F_{(2,38)} = 3.9$; $P = 0.03$ |
| **Tissue N content** | $F_{(2,34)} = 0.4$; $P = 0.7$ | $F_{(2,34)} = 2$; $P = 0.18$ | $F_{(2,34)} = 1$; $P = 0.3$ |
| **Tissue C content** | $F_{(2,34)} = 1$; $P = 0.4$ | $F_{(2,34)} = 0.1$; $P = 0.7$ | $F_{(2,34)} = 1$; $P = 0.6$ |

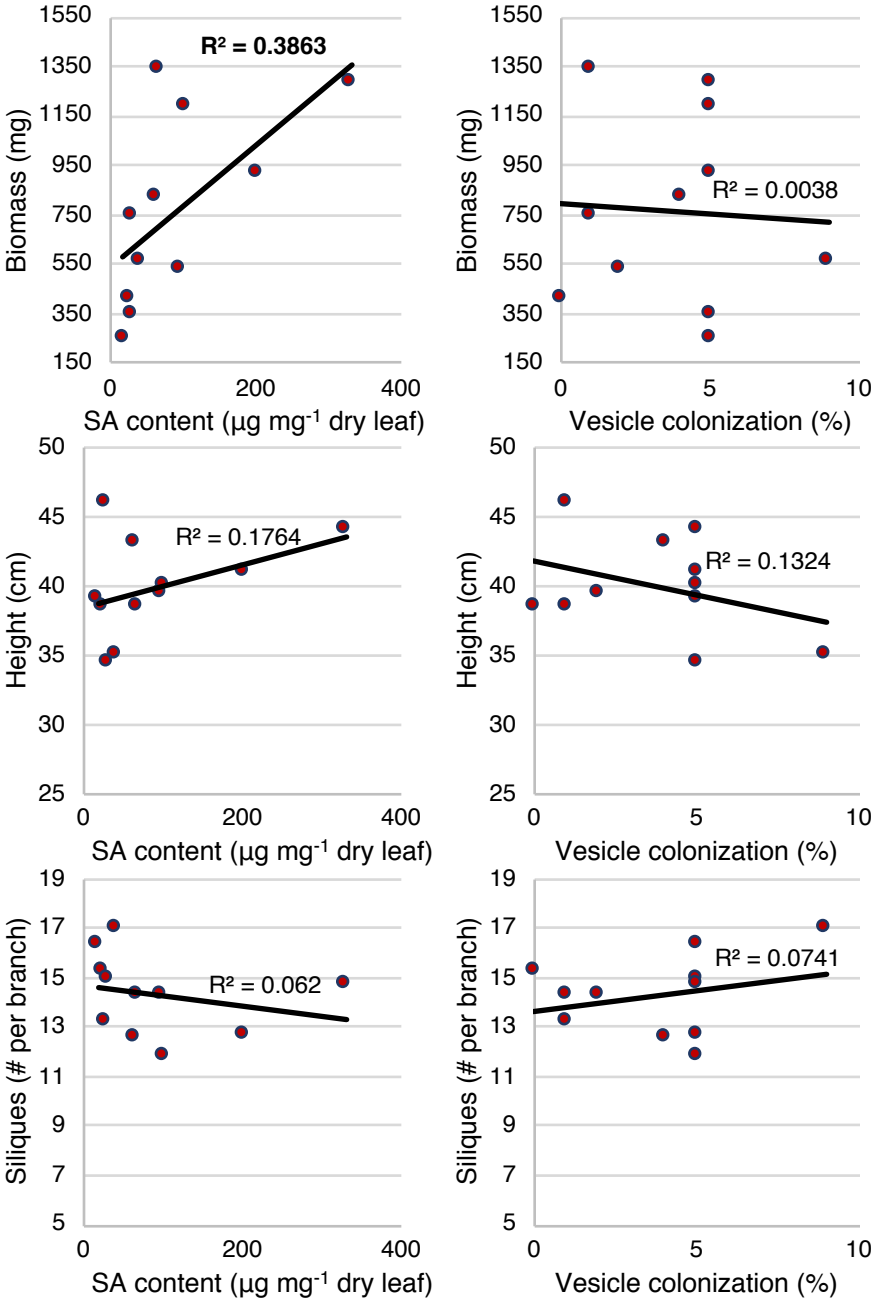
**Supplementary Table 3.2.** Mixed effects model outputs comparing the effects of genotype (IG-removed, wild-type, IG-enhanced), inoculation (sterile vs. AMF), and their interaction.

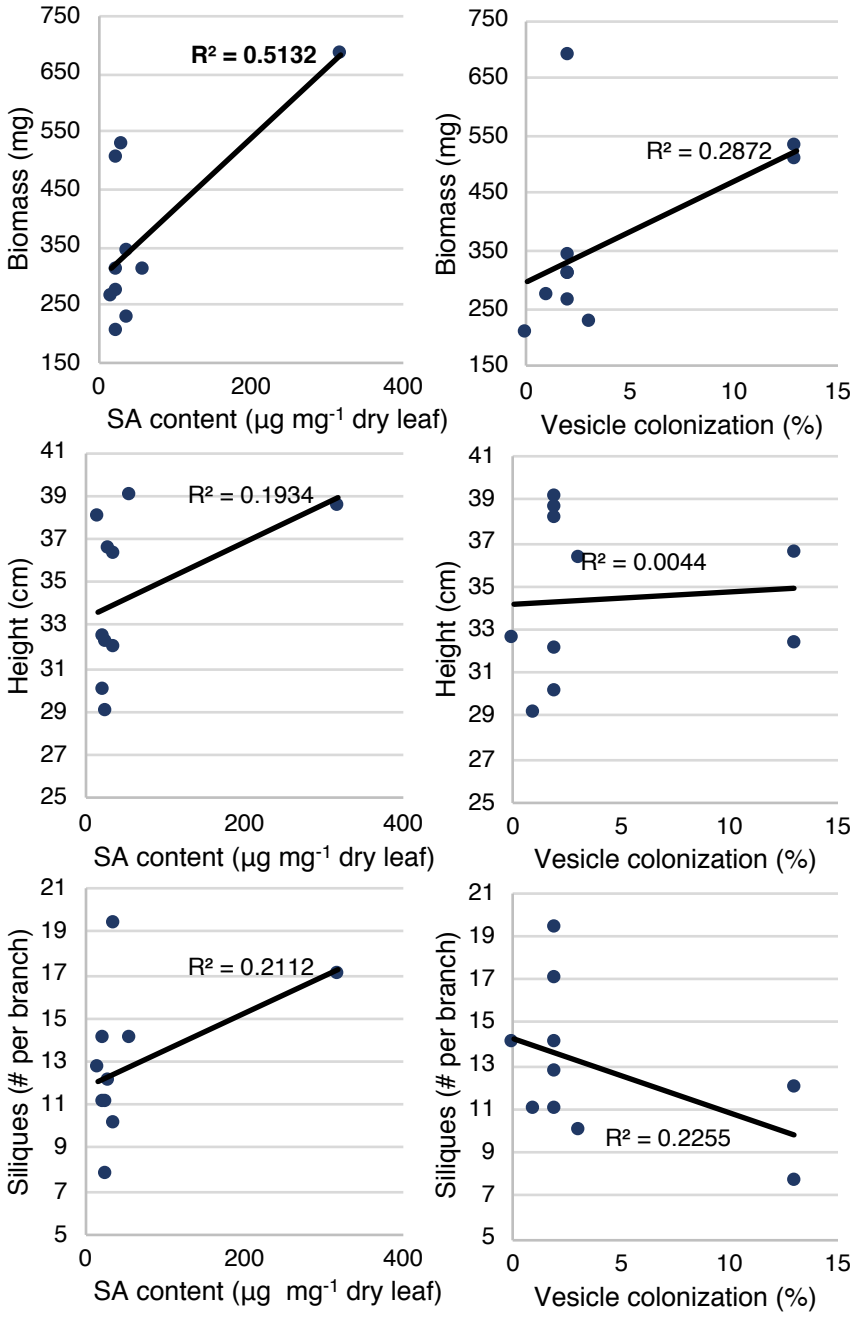
| | Genotype | Inoculation | Genotype × AMF addition |
|---|---|---|---|
| **Salicylic acid** | $F_{(2,37)} = 10$; $P = 0.005$ | $F_{(1,37)} = 37.4$; $P = 0.01$ | $F_{(2,37)} = 1.8$, $P = 0.2$ |
| **Vesicle colonization** | $F_{(2,30)} = 5$; $P = 0.01$ | $F_{(1,30)} = 5$; $P = 0.04$ | $F_{(2,30)} = 0.7$, $P = 0.5$ |




**Supplementary Figure 3.1.** Vesicles (V), an arbuscule (A) and hyphae (H) on *Arabidopsis thaliana* roots. Images were taken at 100x magnification.


Biomass (mg)
SA content (µg mg-1 dry leaf)
R² = 0.0386
Biomass (mg)
Vesicle colonization (%)
R² = 0.3078
Height (cm)
SA content (µg mg-1 dry leaf)
R² = 0.8394
Height (cm)
Vesicle colonization (%)
R² = 0.1115
Siliques (# per branch)
SA content (µg mg-1 dry leaf)
R² = 0.5055
Siliques (# per branch)
Vesicle colonization (%)
R² = 0.0548


R² = 0.3863
Biomass (mg)
SA content (µg mg-1 dry leaf)
R² = 0.0038
Vesicle colonization (%)
R² = 0.1764
Height (cm)
R² = 0.1324
R² = 0.062
Siliques (# per branch)
R² = 0.0741




**Supplementary Figure 3.2**. Correlations between silique production, plant height, and aboveground biomass with SA content and AMF vesicle colonization in the IG-removed (yellow), wild-type (red), and IG-enhanced (dark blue) genotypes. Significant linear regressions have a bolded $R^2$ value.

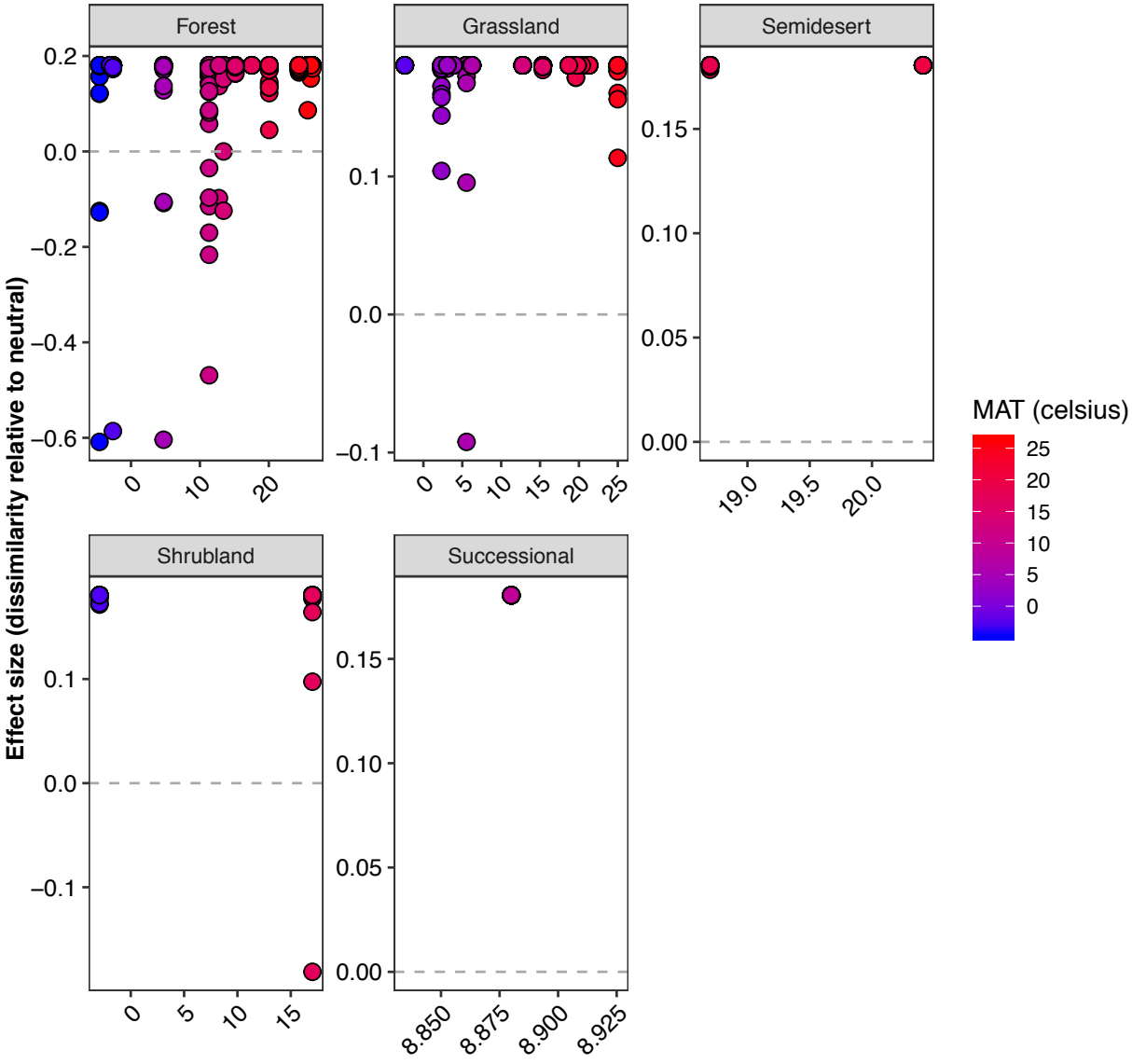
**Supplementary Table 4.1.** The relationship between effect size (Bray-Curtis) and environmental parameters for the entire fungal community at sites across the globe. When there was a significant linear correlation, we compared linear and non-linear fits that explained the highest amount of variation and report these values alongside the linear regression output. We included model variables that were multicollinear with the strongest predictor variables. Bolded predictors show the strongest predictors of effect size.

| | **Linear R2** | **Linear P-value** | **Non-linear model type** | **Non-linear R2** | **Non-linear P-value** | **Multicollinear variables (where *P* > 0.06)** |
|---|---|---|---|---|---|---|
| Longitude | 0.02 | 0.03 | - | - | - | - |
| **Latitude** | **0.18** | **0.001** | **loess** | **0.57** | **< 0.001** | **-** |
| Altitude | N.S. | - | - | - | - | - |
| Forest age | 0.01 | 0.03 | - | - | - | - |
| Tree basal area | 0.04 | 0.0001 | - | - | - | - |
| Site size | NS | - | - | - | - | - |
| EMF tree species | NS | - | - | - | - | - |
| NPP | NS | - | - | - | - | - |
| PET | 0.04 | 0.0001 | - | - | - | MAT |
| **MAP** | **0.07** | **< 0.0001** | **-** | **-** | **-** | **MAT** |
| **MAT** | **0.24** | **< 0.0001** | **binomial** | **0.42** | **<0.0001** | **MAP, PET** |
| Soil moisture | 0.01 | 0.02 | - | - | - | - |
| Soil N contents | NS | - | - | - | - | - |
| Soil C contents | 0.04 | 0.0002 | - | - | - | - |
| **Soil C:N ratio** | **0.2** | **<0.00001** | **-** | **-** | **-** | **-** |
| Delta 15N | 0.03 | 0.003 | - | - | - | - |
| Delta 13C | NS | - | - | - | - | - |
| **Soil pH** | **0.06** | **< 0.0001** | **exponential** | **0.06** | **<0.0001** | **-** |
| Soil P contents | 0.04 | <0.0001 | - | - | - | - |
| Soil K contents | NS | - | - | - | - | - |
| Soil Mg contents | NS | - | - | - | - | - |
| Soil Ca contents | 0.03 | 0.002 | - | - | - | - |

**Supplementary Table 4.2.** The relationship between effect size(Bray-Curtis) and environmental parameters for the fungal community at sites across biomes. Values are significant $R^2$ outputs from linear regressions with non-significant (NS) correlations also designed. There were no significant correlations for grasslands so they are not included.

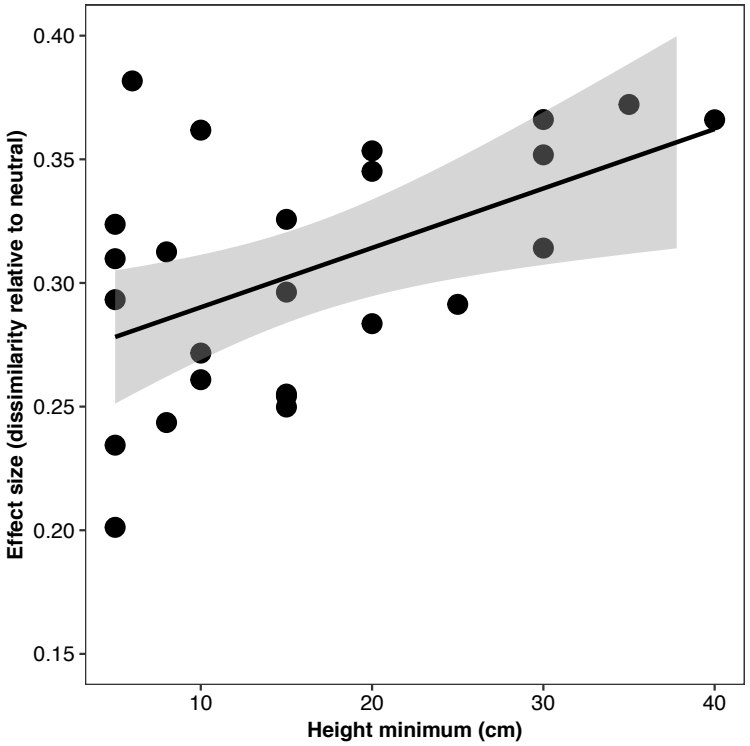
| | Arctic tundra | Boreal forest | T. Con. forest | T. Dec. forest | S. Temp. forest | Dry Trop. forests | Moist Trop. forests | Trop. Mon. forests | Med | Sav |
|---|---|---|---|---|---|---|---|---|---|---|
| Longitude | NS | 0.44 | NS | NS | NS | 0.28 | 0.21 | 0.22 | 0.61 | 0.57 |
| Latitude | NS | 0.6 | 0.22 | 0.3 | NS | NS | NS | NS | 0.7 | NS |
| Altitude | NS | 0.31 | NS | NS | NS | NS | 0.12 | 0.15 | 0.38 | 0.48 |
| Forest age | NS | NS | NS | NS | 0.33 | NS | NS | NS | NS | NS |
| Tree basal area | NS | NS | NS | NS | NS | NS | 0.15 | NS | 0.24 | NS |
| NPP | NS | 0.55 | NS | NS | 0.12 | NS | NS | NS | 0.5 | NS |
| PET | NS | 0.21 | 0.13 | 0.13 | 0.04 | NS | 0.05 | NS | NS | 0.52 |
| MAP | NS | 0.4 | NS | 0.18 | NS | NS | NS | 0.19 | 0.33 | 0.29 |
| MAT | NS | 0.23 | 0.18 | 0.08 | NS | NS | 0.14 | 0.14 | Ns | 0.48 |
| Soil moisture | NS | 0.45 | NS | 0.13 | NS | NS | NS | NS | NS | NS |
| Soil N contents | NS | NS | NS | NS | NS | NS | 0.24 | 0.22 | NS | NS |
| Soil C contents | NS | NS | NS | NS | NS | 0.27 | 0.22 | 0.16 | NS | NS |
| Soil C:N ratio | NS | 0.5 | NS | NS | NS | NS | NS | NS | NS | 0.39 |
| Delta 15N | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Delta 13C | NS | 0.25 | NS | SN | NS | NS | 0.2 | NS | NS | 0.12 |
| Soil pH | NS | 0.44 | NS | 0.21 | NS | NS | 0.2 | NS | 0.26 | NS |
| Soil P contents | NS | NS | NS | NS | NS | NS | 0.18 | NS | 0.5 | NS |
| Soil K contents | NS | NS | NS | NS | NS | NS | 0.2 | NS | 0.32 | 0.14 |
| Soil Mg contents | NS | NS | NS | 0.15 | 0.09 | 0.29 | NS | NS | NS | NS |
| Soil Ca contents | NS | NS | NS | 0.06 | NS | NS | 0.11 | NS | 0.43 | NS |

**Supplementary Figure 4.1.**



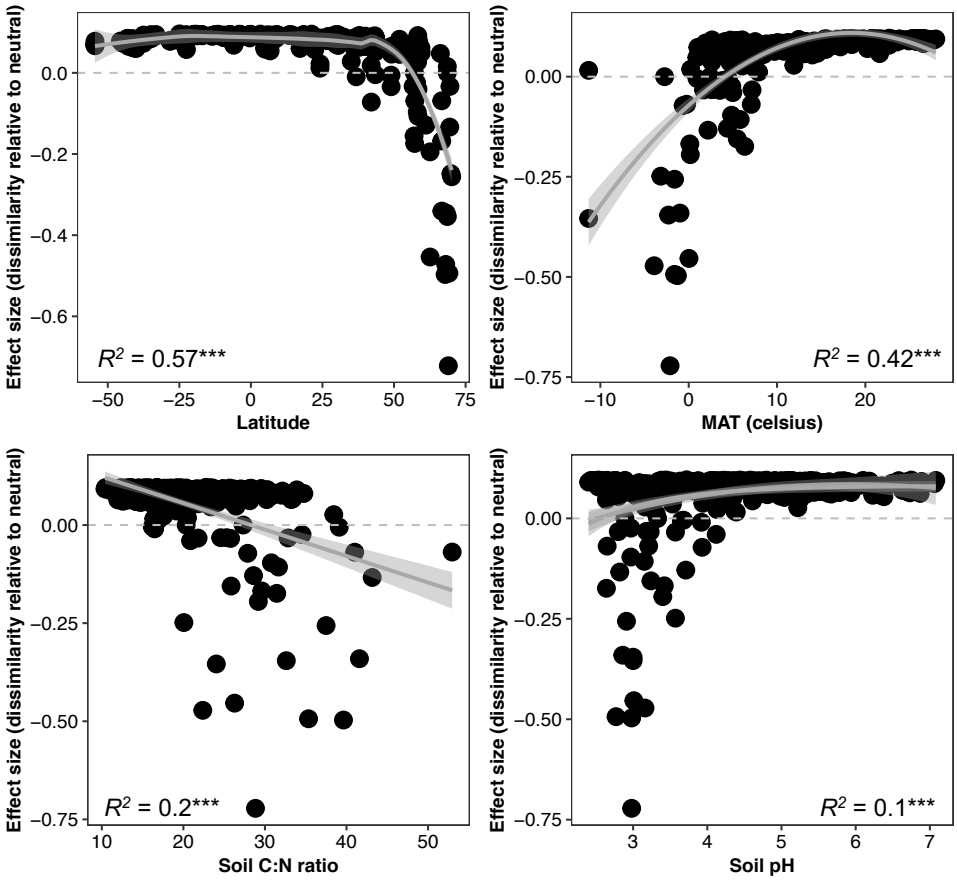

**Supplementary Figure 4.1.** AMF effect (Bray-Curtis) sizes across ecosystem types and mean annual temperature (MAT)

**Supplementary Figure 4.2.**



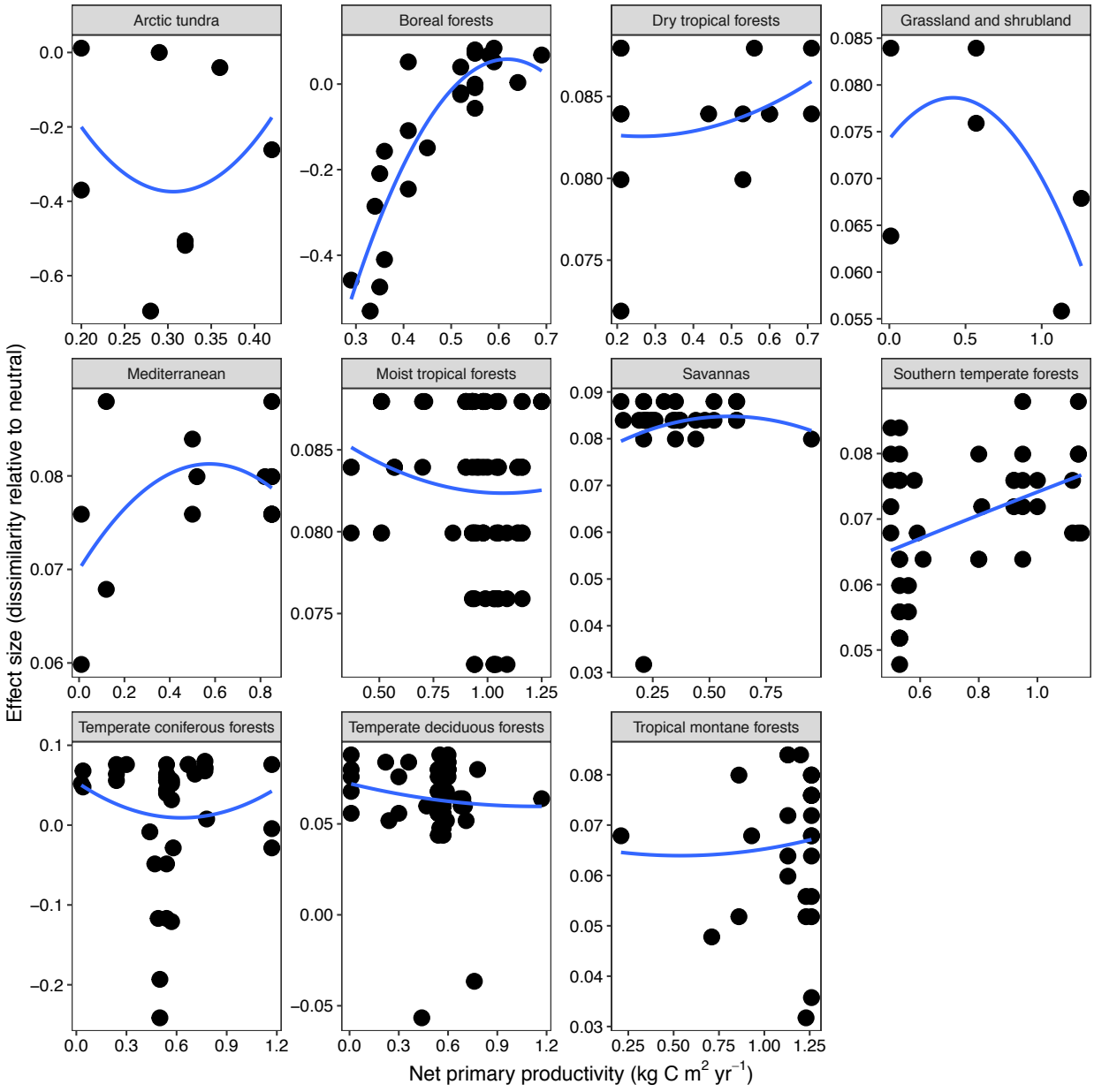

**Supplementary Figure 4.2.** AMF effect size (Bray-Curtis) in relation to host plant height minimum ($R^2 = 0.25$, $P = 0.03$).

**Supplementary Figure 4.3.**



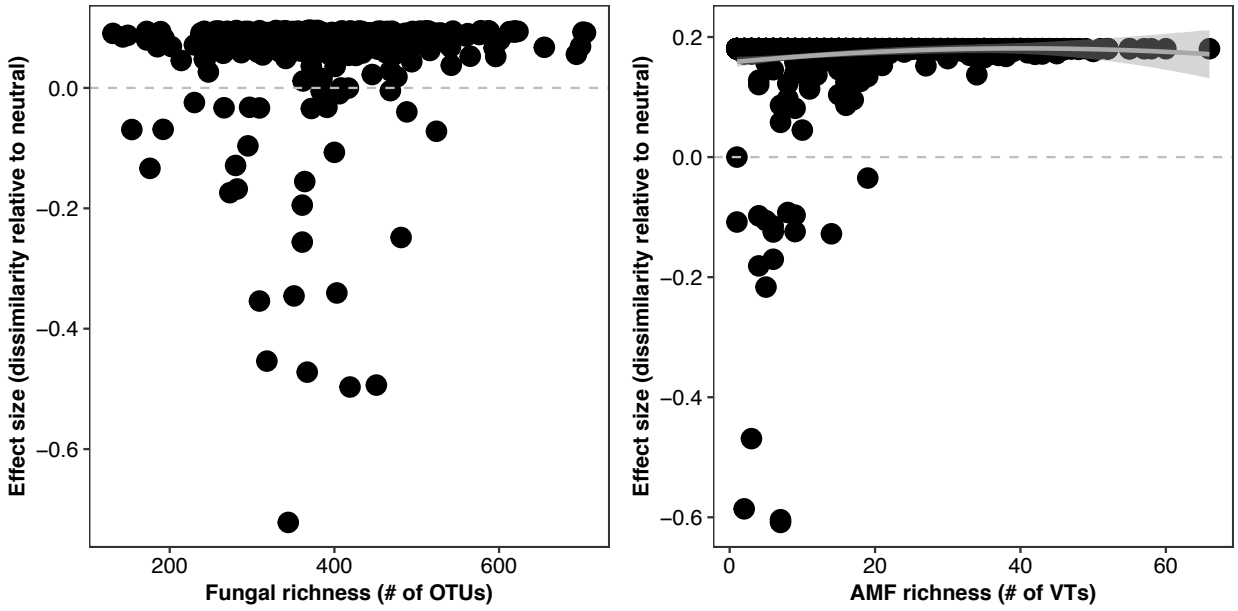

**Supplementary Figure 4.3.** Fungal effect size (Bray-Curtis) in relation to site characteristics that were most significantly correlated (refer to Supplementary Table 1 for a full list of predictor variables and their correlations).

**Supplementary Figure 4.4.**




**Supplementary Figure 4.4.** Fungal effect size (Bray-Curtis) in relation to net primary productivity across biomes.

**Supplementary Figure 4.5.**




**Supplementary Figure 4.5.** Fungal and AMF effect sizes (Bray-Curtis) in relation to fungal and AMF richness. Neither relationships are significant but there is trend for the AMF community.